

**OBESITY INDICES IN CHILDREN WITH  
SPASTIC CEREBRAL PALSY**

by

Daniel Graham Whitney

A dissertation submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Applied Physiology

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SPASTIC CEREBRAL PALSY**

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## ABSTRACT

Cerebral palsy (CP) results from damage or malformation of the developing brain and is the most common physical disability of childhood with approximately 80% of all CP cases having some degree of skeletal muscle spasticity. Children with CP have difficulties with neuromuscular tasks, such as gait, posture and balance, are prone to skeletal muscle weakness and are less physically active than their typically developing peers. Children with CP present with a weak and underdeveloped musculoskeletal system that gets progressively worse throughout growth, potentially increasing the risk for development of musculoskeletal and cardiometabolic diseases. Importantly, mobility and gait function declines as children with CP age into their adult years which may act to exacerbate musculoskeletal pathology, excessive fat accumulation, frailty and chronic disease risk. This is supported by a higher prevalence of age-related chronic diseases and multimorbidity in adults with CP compared to the general population. However, considering age-related chronic diseases often get their roots in childhood, cardiometabolic disease risk and factors associated with cardiometabolic disease risk, such as musculoskeletal health and indices of obesity, have not been examined in children with CP.

The first aim of this dissertation was to determine if ambulatory children with spastic CP have an altered fat distribution of the leg compared to typically developing children using magnetic resonance imaging at the mid-third of the leg. Compared to controls ( $n = 12$ ), children with CP ( $n = 12$ ) had no difference in total ( $p = 0.796$ ) or subcutaneous ( $p = 0.868$ ) fat, but had higher intermuscular ( $p = 0.036$ ) and subfascial ( $p = 0.002$ ) fat and intramuscular ( $p = 0.002$ ) and tibia bone marrow ( $p = 0.004$ ) fat concentration after statistically controlling for tibia length. These findings suggest that

ambulatory children with spastic CP have an altered fat distribution profile of the leg that is favoring musculoskeletal depots as evidenced by no difference in total or subcutaneous fat, but higher fat infiltration of the skeletal muscle and bone marrow depots.

The second aim of this dissertation was to determine the volume and fat concentration profile of the 11 individual leg muscles and how it relates to muscle strength in ambulatory children with spastic CP using magnetic resonance imaging along the length of the tibia and a Biodex dynamometer to assess muscle strength. Compared to controls ( $n = 14$ ), children with CP ( $n = 14$ ) had lower volume of all muscles (all  $p < 0.05$ ), except for the marginally insignificant peroneus longus ( $p = 0.052$ ), ranging from 27 % (peroneus longus) to 44 % (tibialis anterior) and higher intramuscular fat of all muscles (all  $p < 0.05$ ) ranging from 4.4 (tibialis posterior) to 10.1 (soleus) percentile units. The slopes for tibia length regressed on flexor digitorum longus volume and tibialis anterior volume were significantly different between groups (interaction,  $p = 0.040$  and  $0.005$ , respectively). There were positive relationships between plantar flexion strength regressed on gastrocnemius and soleus volume and dorsi flexion strength regressed on tibialis anterior volume in children with CP and controls ( $r^2 = 0.32$  to  $0.71$ , all  $p < 0.05$ ). When intramuscular fat concentration was removed from muscle volume (corrected muscle volume), the relationships became slightly stronger ( $r^2 = 0.37$  to  $0.77$ , all  $p < 0.05$ ). For all the uncorrected and corrected muscle volume-strength relationships, there was a significant difference in the y-intercept between groups, but not the slope. These findings suggest that ambulatory children with spastic CP have smaller muscles that are highly infiltrated with fat and have weaker muscle strength per unit of muscle volume. The higher intramuscular fat

of the gastrocnemius, soleus and tibialis anterior is contributing to muscle weakness in ambulatory children with spastic CP; albeit, to a small extent.

The third aim of this dissertation was to determine if ambulatory children with spastic CP have higher abdominal fat compared to typically developing children using dual-energy x-ray absorptiometry. There were no group differences in body mass index (BMI), BMI % or total body fat mass index (FMI; all  $p > 0.05$ ). Compared to controls ( $n = 18$ ), children with CP ( $n = 18$ ) had higher trunk FMI ( $p = 0.019$ ), abdominal FMI ( $p = 0.001$ ) and visceral FMI ( $p = 0.001$ ) but no difference in subcutaneous FMI ( $p = 0.088$ ). These findings suggest that ambulatory children with spastic CP have a higher concentration of fat in the abdominal region compared to typically developing children, which is not captured by BMI or total body fat.

The fourth aim of this dissertation was to determine if BMI can estimate body composition in children with CP. There were no group differences in BMI or BMI % between all children with CP ( $n = 42$ ) and controls ( $n = 42$ ) or among nonambulatory ( $n = 18$ ) and ambulatory ( $n = 24$ ) children with CP and controls (all  $p > 0.05$ ). Compared to controls, nonambulatory and ambulatory children with CP had higher FMI ( $p < 0.001$  and  $0.019$ , respectively) and lower fat-free mass index (FFMI;  $p < 0.001$  and  $0.036$ , respectively) after statistically controlling for BMI. BMI was a strong predictor of FMI and a moderately-strong predictor of FFMI in children with CP ( $r^2 = 0.59$  and  $0.47$ , respectively, both  $p < 0.001$ ). The prediction of FMI ( $R^2 = 0.86$ ) and FFMI ( $R^2 = 0.66$ ) from BMI significantly increased (both  $R^2$  change,  $p < 0.05$ ) when age, sex and ambulatory status (i.e., either ambulatory or nonambulatory) were included in the regression model. These findings suggest that children with CP have a higher FMI and lower FFMI for a given BMI which is more pronounced in nonambulatory than

ambulatory children with CP. Importantly, the prevalence of obesity may be even higher than reported. Fortunately, this aim developed validated statistical models to estimate FMI and FFMI from easily attainable measures.

The overall findings from this dissertation provides a comprehensive assessment of obesity indices in children with CP that often precede cardiometabolic disease. These findings suggest that children with CP are at a very high risk for an early and accelerated development of age-related chronic diseases. Future efforts are needed to monitor changes in these fat depots with age or in response to interventions and how these higher obesity indices related to cardiometabolic disease risk in children with CP.

## **Chapter 1**

### **INTRODUCTION**

Cerebral palsy (CP) results from damage or malformation of the brain around the time of birth and is the most common physical disability in childhood affecting 3.1 per 1000 live births [1]. The brain damage associated with CP is more commonly a result of hypoxic-ischemia encephalopathy, intraventricular hemorrhage, periventricular leukomalacia or cerebral dysgenesis [2]. In the past, children with more severe forms of the disorder tended to not survive past their childhood years. However, over the past five decades, the survival rate has been growing where today, approximately 87% of children with CP are living to at least 30 years of age [3]. Since the turn of the second millennium, almost one million individuals in the USA have some form of CP [4] with the vast majority (~80 %) having muscle spasticity [5]. In parallel with the increasing survival rate, there has also been an increase in the prevalence of age-related chronic diseases in the young adult CP population. What is concerning about this phenomenon is that these chronic diseases are presenting in more severe forms and at accelerated rates compared to the general population [6, 7].

The number and magnitude of complications associated with CP seems to be governed by the severity of the disorder. While there are many classification systems to determine the extent of the disorder, when used in combination, gross motor impairment and topographical distribution of affected areas are strong predictors of functional and health outcomes in those with CP. Gross motor impairment is often measured using the Gross Motor Function Classification System (GMFCS); a scale

ranging from I - V coinciding with mild to severe forms [8]. Briefly, children classified as I and II are independent ambulators; those classified as III often rely on assistive walking devices for mobility; those classified as IV primarily use manual wheelchairs for mobility; those classified as V are wheelchair bound. The anatomical distribution of spasticity is heterogeneous presenting as hemiplegic, diplegic, quadriplegic or a unique mixture of the three presentations and affects the extremities and trunk in a distal to proximal direction.

### **1.1 Diminished Motor Control**

The corticospinal tract is a bundle of nerves originating from the cerebral cortex and is responsible for volitional motor control. In healthy children, the corticospinal tract progressively matures up to the age of 13 years [9] which is driven by postnatal primary motor cortex activity [10]. Damage to the central nervous system, resulting in CP, often disrupts the development [11, 12] and maturation [13, 14] of the corticospinal tract resulting in decreased voluntary activation, greater coactivation of antagonists [15, 16] and reduced neuromechanics [17] during volitional movements, leading to motor dysfunction such as disturbances in gait, posture and balance and weak muscle strength; all of which are hallmark presentations of those with CP.

The resulting milieu of motor dysfunction helps to explain, in part, why children with CP are less physically active compared to typically developing children [18-20]. Specifically, children with CP spend more time in sedentary behavior [20, 21] and less time in moderate-to-vigorous physical activity [21]. Low levels of physical activity and low participation likely minimizes motor-related sensory feedback to the central nervous system. This may exacerbate the motor dysfunction observed in young children with CP due to a lack of stimulus of the primary motor cortex [10]. This is supported

by the mobility and gait decline as children with CP age into their adult years [22]. The combination of neuromuscular dysfunction and a lack of mechanical loading during growth results in a lower accretion of muscle and bone.

## **1.2 Musculoskeletal Health**

Poor musculoskeletal health in adults is associated with poor mobility [23, 24], lower levels of physical activity [25, 26], increased risk of chronic disease development [27-29] and early mortality [30, 31]. Factors influencing the health of the adult musculoskeletal system have the greatest impact during childhood. Therefore, inadequate accretion of muscle and bone during childhood has the potential to accelerate these negative health-related consequences. Indeed, those with CP have a greater risk of developing age-related chronic diseases more rapidly when transitioning to adulthood [6, 7], due, in part, to a weak and underdeveloped musculoskeletal system that is already present in childhood [32-34] with the underdevelopment present in children as young as 3 years of age [35]. The functional decline seen in those with CP as they age into their adult years [22] can lead to excessive fat accumulation and exacerbate frailty and the development of age-related chronic diseases.

Excessive abdominal and total body fat is associated with increased risk of cardiometabolic disease. However, excessive fat accretion in the musculoskeletal system vs. total body fat may have a stronger influence on muscle function [36] and cardiometabolic disease risk in populations with reduced mechanical loading and altered body composition [27, 28], such as those with CP. Ambulatory adults with mild CP have a greater fat content within the plantarflexor muscles compared to healthy adults [37]. Nonambulatory children with more severe forms of CP have a greater infiltration of skeletal muscle fat tissue that is negatively correlated with physical

activity [18]. While skeletal muscle fat infiltration and cardiometabolic disease risk have not been assessed together in the CP population to date, studies of other human populations have found a strong influence of intramuscular fat content and cardiometabolic disease risk factors [27, 38-40].

Bone marrow fat accumulation is prevalent in the aging process and in those with skeletal disease [41-44]. In a study of healthy and diabetic pigs, vertebral bone marrow fat content was inversely related to vertebral bone marrow glucose uptake and utilization ( $r^2 = 0.64, p < 0.01$ ) [45]. In a study of postmenopausal women, vertebral bone marrow fat content was found to be positively related to fasting glycated hemoglobin (a more stable measure of long term glucose load) in diabetics ( $r^2 = 0.68, p < 0.05$ ), but not in nondiabetics [46]. Furthermore, when they split the diabetic women into two groups that either fell below or above 7 % glycated hemoglobin levels, a target value below 7 % improves microvascular function in adults with diabetes [47], the diabetics above 7 % had greater vertebral bone marrow fat content than the diabetics below 7 % ( $p < 0.05$ ) [46].

Taken together, the health of the musculoskeletal system and its fat profile has an important regulatory function on mobility and mitigating excess body fat and the development of chronic diseases. Children with CP have a progressively underdeveloping musculoskeletal system, and when transitioning into adulthood, are suffering from a variety of age-related chronic diseases. Therefore, there is a need to investigate factors, such as musculoskeletal, abdominal and total body fat, that contribute to cardiometabolic disease risk during growth and development in children with spastic CP.

## **Chapter 2**

### **LITERATURE REVIEW**

#### **2.1 Motor Dysfunction**

Cerebral palsy (CP) results from damage or malformation of the infant brain leading to neuromuscular complications such as dysfunctions in gait, posture and balance, muscle spasticity and skeletal muscle weakness [32].

##### **2.1.1 Neuromuscular Dysfunction**

The maturation of the primary neuromotor pathway is blunted in children with CP as evidenced by a reflex modulation pattern in teenagers with CP that is similar to typically developing children [13]. Altered peripheral neural input to the working muscles have been noted. During volitional movements, children with CP have lower neural drive to the agonists and higher neural drive to the antagonists, or coactivation, compared to typically developing children [15, 16]. Further, evidence of impaired neuromechanics in children to adults with CP compared to age-matched controls has been reported [17].

Skeletal muscle tissue adapts its mechanical function and morphology according to spatial and temporal characteristics of neural input [48]. The altered peripheral neural drive to the musculature in children with CP is associated with disruptions in the microanatomy of the neuromuscular junction [49]. Acetylcholine, a neurotransmitter, in the neuromuscular junction is responsible for relaying the action potential from the neuron to the myocyte to elicit muscle contraction. Acetylcholine

receptors were found to be located outside of the functioning portion of the neuromuscular junction in children with CP but not in typically developing children [50], providing further evidence of neuromuscular dysfunction in terms of morphology.

### **2.1.2 Gross Motor and Spastic Muscle Function**

Children with CP are less physically active regardless of ambulatory ability [18, 51]. Specifically, children with CP spend more time in sedentary behavior and less time in total, light, moderate and vigorous activity [21] which is associated with lower cardiorespiratory fitness and cardiometabolic health in this population [52]. Children with spastic CP have greater energy expenditure while walking which is related to gait parameters of the lower extremities [53]. In hemiplegic children and adolescents with spastic CP, the concentric work from the ankle plantar flexors, knee extensors and hip extensors and flexors during ambulation was 19 - 51 % lower in the involved vs. less involved side [54].

The lowered ambulatory and gross motor function capability may be explained by muscle spasticity [55, 56]. However, muscle spasticity was reported to explain only 27 % of the variance in measures of gross motor function in nonambulatory children with CP [57]. To better understand factors that impact gross motor function and functional outcome, Kim et al. [58] employed path analysis in 81 ambulatory and nonambulatory children with CP. The path analysis method determines causal relationships between a set of variables providing an explicit theory of relationships rather than testing a set of data from a linear relationship. The authors found that both muscle spasticity and muscle strength are causal factors in the functional outcome of children with CP through their negative effects on gross motor function. Specifically, muscle strength ( $\beta = 0.447$ ,  $p < 0.05$ ) was a stronger direct predictor of gross motor

function than was muscle spasticity ( $\beta = -0.339$ ,  $p < 0.05$ ) and a stronger indirect predictor of functional outcome as assessed by the Functional Skills domain of the Pediatric Evaluation of Disability Inventory ( $\beta = 0.317$  vs.  $\beta = -0.240$ , both  $p < 0.05$ ).

Children with CP have weaker muscles [32] that is more severe in the distal vs. proximal musculature [15]. Muscle strength plays an important role in ambulation [53, 54, 59, 60] and gross motor function [58, 61-63] in children with CP. While maximal force production typically takes up to 300 ms in most human muscles [64], most everyday activities, such as gait, transfers and other higher levels of activities, require limb movements involving contraction times of 50 - 200 ms [65]. Therefore, rate of force development, or, temporal characteristics of submaximal force generation, may prove to be a better indicator of gross motor function in those with neuromuscular disease. Moreau et al. [66] reported 50% lower maximal voluntary isometric contraction (MVIC) and 70% lower rate of force development in the knee extensors in ambulatory children with CP compared to typically developing children. These two measures were not correlated with one another in children with CP ( $r^2 = 0.26$ ,  $p > 0.05$ ) suggesting different mechanisms of action, and therefore, potentially different roles in motor function. Indeed, rate of force development measures were a better predictor of functional mobility, such as transfer ( $r^2 = 0.40 - 0.48$ ,  $p < 0.05$ ) and sport ( $r^2 = 0.62 - 0.67$ ,  $p < 0.05$ ) measures than MVIC ( $r^2 = 0.10$ ,  $p = 0.34$ ;  $r^2 = 0.49$ ,  $p < 0.05$ , respectively) in children with CP.

Although the injury associated with the development of CP occurs initially in the central nervous system, symptoms are commonly treated at the level of the muscle [67]. Muscle tissue is highly plastic to the stimulus it receives, especially during growth. The accretion, mechanical function and health of the musculature is predominantly driven

by postnatal development of mechanical stresses imposed on the musculature [68]. The majority of children with CP have muscle spasticity, are less physically active, have a lower force generating capacity and a slower rate at which they can produce force. These factors negatively impact muscle morphology. Muscle architecture and quality play an important role in functional and health outcomes in those with CP, especially during growth and development.

## **2.2 Spastic Muscle**

Several studies report the importance of lower extremity muscle strength [59-61, 63], time-dependent measures of force production [66] and muscle architecture [54, 66] on gross motor function in children with CP.

### **2.2.1 Spastic Muscle Architecture**

Although there are a limited number of studies of muscle architecture measures in those with CP [69], there is general agreement that children and adolescents with CP have smaller muscles of the lower extremities compared to typically developing children [70-73], the distal musculature of the lower extremities is smaller than the proximal musculature [70, 74], the involved vs. the uninvolved lower extremity musculature is smaller and weaker in hemiplegic CP [54, 74] and muscle fascicle [66] and belly length [73] may be different in children with CP compared to typically developing children.

The muscle size-strength relationship observed in the healthy population [33, 75-77] has been demonstrated in children with CP. However, this relationship is weaker in children with CP due to a greater deficit in muscle strength (63%) vs. muscle volume (53%) in the knee extensors and flexors [33]. As previously mentioned, Moreau and

colleagues [66] reported that children with CP had a lower rate of force development of the knee extensors compared to typically developing children. In both groups, rate of force development was associated with muscle thickness of the vastus lateralis, although, the relationship was weaker in children with CP ( $\beta = 18.49$ ,  $p = 0.041$ ) compared to typically developing children ( $\beta = 26.04$ ,  $p = 0.002$ ). Moreover, rate of force development was also associated with fascicle length and fascicle angle of the vastus lateralis and rectus femoris in addition to the cross sectional area of the rectus femoris in typically developing children ( $r = 0.68 - 0.84$ ; all  $p < 0.05$ ), but not in children with CP.

### **2.2.2 Spastic Muscle Quality**

Muscle architecture does not fully explain the force generating capacity of muscle in children with spastic CP. Muscle quality may be a better indicator of muscle function [78]. Muscle quality includes intramuscular contractile properties and intramuscular noncontractile tissue. In a rat model of acute unloading, the semimembranosus muscle exhibited decrements of force normalized to muscle size that was associated with a decrease in the quality of intramuscular contractile tissue [79]. The plantar flexor strength deficits observed in adults with type II diabetes mellitus (T2DM) cannot be explained by muscle size, but rather, by a significant increase in intramuscular noncontractile tissue [80]. Spastic musculature undergoes changes to the intramuscular contractile properties and is associated with an increase in intramuscular noncontractile tissue [37].

### 2.2.2.1 Intramuscular Contractile Properties

In an attempt to characterize alterations of spastic muscle in regards to major muscle physiological systems in children with CP compared to typically developing children, Smith and colleagues [81] employed DNA microarray analysis and found over 200 genes to be altered in wrist muscles from children with CP. In the excitation-contraction coupling system, altered calcium handling was noted as evidenced by gene disparities leading to chronically increased intramyocellular calcium levels and therefore altering contraction capability. Myosin heavy chains are largely responsible for fiber type determination [82] and when transitioning, due to mechanical and biochemical factors, will do so in a slow-to-fast phenotype. In the fiber type determination system from the study by Smith and colleagues [81], genes encoding myosin heavy chains relating to type IIb and IIx fibers (fast fibers) were upregulated while the gene encoding type I fiber (slow fiber) myosin heavy chain was downregulated. This slow-to-fast transition of muscle fibers is in agreement with other studies using immunohistochemical techniques and found spastic muscles to be predominately fast fibers [83-85] with greater variability in fiber size and shape [86], lower oxidative capacity and less fatigue-resistant, potentially explaining muscle weakness in those with muscle spasticity [83]. In children with CP, the extent of fast fiber predominance is associated with neurogenic atrophy and is significantly related to the number of botulinum toxin injections ( $r^2 = 0.79, p < 0.001$ ) [85]; ironically, a routine treatment to temporarily reduce muscle spasticity with effects lasting about 6 months.

In the myogenesis system [81], the majority of pathway elements were unchanged except for the upregulation of genes encoding insulin-like growth factor (*IGF1*) and myostatin (*GDF8*) which have opposing effects on muscle growth; IGF1 enhances muscle growth whereas myostatin inhibits muscle growth. One of the anabolic

effects of IGF1 is to increase the number of extracellular matrix proteins [87, 88]. In the study by Smith and colleagues [81], genes encoding protein components of the extracellular matrix were upregulated which is consistent with a report of excessive accumulation of collagen in the muscle extracellular matrix which was positively associated with spasticity [89] and muscle bundle [90] and fiber [91] stiffness in children with CP. Taken together, one can postulate that while the genes encoding IGF1 and myostatin were both increased, the former may be acting to increase the extracellular matrix and thereby indirectly decreasing the functional component of the muscle fiber (i.e., increasing stiffness) while the latter is inhibiting muscle growth via an endocrine mechanism. This interesting display of the myogenic transcriptional profile is unique to CP when compared to other spastic muscle pathology models [81, 92].

A later study by Smith and colleagues [93] using the same DNA microarray analysis found similarities and differences with alterations of major muscle physiological systems in spastic gracilis and semitendinosus compared to their previous study of the wrist muscles from children with CP compared to typically developing children [81]. The primary findings from their later study was that the lower extremity spastic muscles exhibited lower metabolic capability and protein turnover and dramatic increases of extracellular matrix which was negatively associated with muscle fiber bundle stiffness [93]. The few differences in the transcriptional profile of spastic muscle between their studies could have been due to the difference in muscles studied. Wrist muscles will behave and respond differently than muscles of the hip and thigh due to differences in structure and mechanical roles, such as weight-bearing and anti-gravity functions. Furthermore, the first study by this group compared 6 children with CP vs.

2 typically developing children whereas the later study had more subjects (n = 10/group).

This group continued their pursuit in identifying mechanisms of altered muscle physiology in spastic muscles from children with CP. Using flow cytometry from muscle biopsies of spastic gracilis and semitendinosus, Smith et al. [94] concluded that children with CP have a lower proportion of satellite cells compared to typically developing children. Satellite cells are the main constituents of muscle stem cells and are responsible for the growth and repair of the musculature, suggesting that children with CP have a diminished myogenic capacity. This becomes important during development because the spastic muscle profile in children with CP outlined above may explain the inadequate accretion of longitudinal muscle growth. In addition, this profile likely leads to a blunted response from rehabilitation efforts aimed towards enhancing muscle size, such as strength training, in children with CP.

#### **2.2.2.2 Intramuscular Fat**

Skeletal myocytes sequester plasma free fatty acids and converts them to intramuscular triacylglycerol (TG) to undergo  $\beta$ -oxidation for fuel metabolism [40, 95-97]. In times of low energy demand (e.g., physical inactivity), intramuscular TG gets stored in lipid droplets [40]. Deleterious effects of increasing intramuscular fat on muscle function and structure are noted below.

Adults with chronic obstructive pulmonary disease (COPD) have low levels of physical activity [98], smaller muscles [99] and greater intramuscular fat content [100] in the lower extremities compared to the general population. Robles et al. [101] demonstrated that intramuscular fat content of the lower extremity is a stronger predictor of muscle strength and ambulatory ability than muscle size in those with COPD ( $r^2 = -$

0.49 to -0.64,  $p < 0.001$  vs.  $r^2 = 0.15$  to  $0.49$ ,  $p < 0.05$ , respectively). Reports in older adults suggest that high levels of intramuscular fat content is associated with balance and ambulation dysfunction [23, 102], attenuation of muscle quality and function in response to longitudinal exercise intervention [103] and increases in intramuscular inflammation that is exacerbated by low levels of physical activity [28]. Adults with T2DM have a lower plantar flexor force generating capacity despite not having differences in plantar flexor muscle volume. The reported loss of strength is associated with greater intramuscular fat content [80]. In a study investigating vastus lateralis muscle fiber function and intramyocellular lipid (IMCL) deposition (40 slow fibers averaged per participant) in older normal weight (i.e., controls;  $n = 13$ ) and obese ( $n = 21$ ) adults, Choi and colleagues [36] discovered that the slow fibers in the obese group had a 9 % greater cross sectional area and a two-fold greater IMCL area compared to controls. The obese group also had lower specific force (i.e., force normalized to muscle fiber cross sectional area) and power generating capacity of the muscle fibers. Moreover, IMCL area and number were inversely related to the lower power generating capacity of the muscle fibers in the obese group ( $r^2 = 0.48 - 0.56$ , all  $p < 0.05$ ) but not in controls ( $r^2 = 0.14 - 0.40$ , all  $p > 0.05$ ).

Using magnetic resonance imaging, Johnson et al. [18] reported that nonambulatory children with CP had smaller thigh muscles and elevated subfascial and intermuscular adipose tissue that was negatively related to physical activity ( $r = -0.40$  and  $-0.58$ , respectively, both  $p < 0.05$ ) while Noble et al. [37] reported that ambulatory adults with spastic CP had smaller leg muscles and a greater intramuscular fat content in the plantar flexors compared to typically developed adults. These data suggest that intramuscular fat indirectly impairs muscle function by accumulating at the expense of

contractile tissue. In a more direct physiological role on muscle function, DNA microarray analysis from the semimembranosus of female pigs revealed that greater intramuscular fat content was associated with downregulation of myofibril proteins; specifically, actin 3, nebulin and titin [104]. On the other hand, myofibril proteins, such as titin, are not altered in spastic muscles [105, 106]. Taken together, for a given muscle or muscle fiber size, intramuscular fat accumulation will correspond with reduced contractile tissue and may alter the gene expression of myofibril proteins involved in muscle fiber contraction ultimately leading to muscle weakness; a complication prevalent in those with CP [66].

#### **2.2.2.2.1 Intramuscular Fat Depots**

Intramuscular fat is a composite of intramyocellular lipid (IMCL) and extramyocellular lipids (EMCL). The majority of studies that discern between the two skeletal muscle fat depots typically investigate IMCL and therefore, EMCL is not as well understood in its relation to muscle function and systemic energy metabolism. In any case, as noted above, muscle weakness may contribute to the lower levels of physical activity observed in those with CP. Physical inactivity alters molecular pathways by downregulating proteins that are responsible for sequestering fatty acids towards intramyocellular depots. However, increases in intramuscular fat are still observed [107], suggesting a partitioning of fatty acids towards storage as EMCL. Using magnetic resonance spectroscopy, Jonkers et al. [108] reported that paraplegic adults displayed 4.6-fold elevated EMCL and no difference in IMCL compared to controls. Shah et al. [109] reported similar EMCL findings in spinal cord injured adults, but this group also displayed 3.3-fold elevated IMCL compared to controls. The difference in the time-since-injury and onset of physical inactivity between the studies

( $10.0 \pm 2.6$  vs.  $1.4 \pm 0.8$  years, respectively) may suggest a time-dependent adaptive mechanism of fatty acid partitioning favoring EMCL. This is because IMCL serves as an immediate energy reservoir to facilitate myocyte contraction. Chronic physical inactivity, due to a neurological insult, would result in a chronically lower energetic demand from the muscle and therefore dampened reliance on IMCL. In addition, these patients likely had some degree of muscle damage, due to the nature of acquiring a neurological insult, which is associated with an expansion and proliferation of fibro-adipogenic precursor cells that give rise to adipocytes at the expense of myocytes [110] occurring in the extramyocellular space [111]. The unformed myocytes in a muscle-damaged environment is thought to be a result from muscle precursor cells favoring a fibro-adipogenic progeny that would have otherwise given rise to satellite cells [110]; the primary cells responsible for the development and regeneration of myocytes. Indeed, children with CP have a lower population of muscle satellite cells compared to controls [94] suggesting a model of muscle damage in this population.

In summary, the molecular-to-gross tissue changes occurring in the musculature in children with CP include alterations in transcriptional genes involved in major muscle physiological and structural components of muscle fibers, decreased myogenic capacity, increased extracellular matrix protein deposition, altered fiber type distribution, decreased muscle fiber oxidative capacity and fatigability, small and weak muscles and changes in muscle fiber length. Ultimately, this helps explain why gross motor function and the muscle force generating capacity is hindered in children with CP. However, the extent of the deficit in muscle function and muscle response to longitudinal growth or interventions that is attributed to intramuscular fat accumulation in children with CP is unknown.

### **2.3 Skeletal Status in Children with Cerebral Palsy**

Children with CP suffer from a high incidence of low-energy fracture with a fracture distribution of approximately 80 % occurring in the lower extremities [112]. This is the opposite of healthy children where approximately 80 % of fractures occur in the upper extremities [113]. The fracture susceptibility and unique distribution in children with CP may be explained by a weak and underdeveloped skeleton, especially in the lower extremities [18, 34, 114, 115], that becomes worse throughout growth and development [114]. Using magnetic resonance imaging, Modlesky et al. [34] demonstrated that nonambulatory children with CP present with a severely underdeveloped mid-femur as indicated by a 51 – 55% lower total, cortical and bone marrow cavity volume, thinner cortical walls and 60 – 71% lower estimates of bone strength compared to typically developing children [34]. This skeletal underdevelopment is also present at the distal femur, the primary fracture site in children with CP [112], as evidenced by a low concentration of trabecular bone microarchitecture [115, 116]. What's more concerning is that the pattern of trabecular bone microarchitecture deficit becomes more pronounced with distance from the growth plate in the metaphyseal region of the distal femur in nonambulatory children with CP compared to typically developing children [115], explaining why this site is subject to such a high rate of fracture in this population [112]. Moreover, bone mineral density, which gives the strength and rigidity of bone on a material level, has been found to be lower in children with CP [114, 117]. Taken together, children with CP have poor skeletal health in terms of bone geometry, strength and material properties resulting in a high rate of low-energy fracture. This becomes clinically important because fractures limit mobility in children with CP who already have low levels of mobility and also increases the likelihood of experiencing further fractures [112].

### **2.3.1 Bone Turnover Models for Children with Cerebral Palsy**

Spinal cord injury is often used as a model to understand the pathophysiology of the skeletal system in those with CP [118, 119]. Evidence from adults with spinal cord injury suggests that the substantial bone loss observed during acute skeletal unloading after injury [120-124] is more related to elevated bone resorption [125] while chronic unloading is more related to suppressed bone formation [126]. Rett syndrome is a neurodevelopmental condition presenting in early development (< 18 months of age) with somewhat similar growth, motor [127, 128] and musculoskeletal deficits [129, 130] as seen in children with CP. In an animal model of Rett syndrome, bone histomorphometry of the femur revealed a decreased number of osteoblasts, altered osteoblast morphology and function and a similar number of osteoclasts when compared to wild type mice [131]; a bone biology phenotype more in line with the chronic unloading bone turnover seen in adults with spinal cord injury. The altered bone biology found in the mouse model of Rett syndrome is in accordance with a study of girls with Rett syndrome [132]. Total body bone mineral deficit was observed in girls with Rett syndrome, aged 1 - 17 years, concomitant with a suppressed serum marker of bone formation (i.e., osteocalcin) when compared to an age-matched reference group of typically developing girls. On the other hand, there were no group differences in a serum markers of bone resorption (i.e., C-telopeptide) except for the age group of 5 - 9 years where girls with Rett syndrome had elevated levels of this bone resorption marker. The bone turnover profile is somewhat similar between adults with chronic spinal cord injury and girls with Rett syndrome; two clinical models similar to CP in terms of skeletal status. However, to date, the bone turnover profile in children with CP is unknown. The dynamic and atypical bone turnover profile observed in adults with SCI, which is influenced by time since injury (i.e., acute vs. chronic), and in those

with Rett syndrome may not accurately reflect the bone turnover profile in children with CP due to differences in the timing of neurological injury [34, 133, 134] and the neurodevelopmental nature of Rett syndrome, but may provide evidence of bone turnover metabolism in children with CP. This becomes particularly important because of the interest in using anti-resorptive drugs to enhance skeletal accretion in children with CP [135]. These drugs have known side effects that could be deleterious to a developing child and, based on the evidence from the above clinical models, may not be targeting the key biological components speculated to be driving the underdevelopment of the skeletal system. More work is needed in this area of clinical investigation.

### **2.3.2 Endocrine Control of Bone**

Skeletal health is dictated by the coupling of osteoblast-mediated formation and osteoclast-mediated resorption; a coupled process that is regulated at the endocrine level. Parathyroid hormone is released from the parathyroid glands when serum calcium is low and acts on the kidneys, intestines and skeleton. Intermittent bouts of parathyroid hormone enhances bone mass [136] by activating bone lining cells and promoting bone formation [137] whereas prolonged endogenous parathyroid hormone exposure increases bone resorption [138] leading to transient increases in serum calcium and phosphate; the two most abundant ions that make up the skeletal crystal, hydroxyapatite. Parathyroid hormone also acts to convert the inactive form of vitamin D to its active form, calcitriol, which enables the facilitation of calcium and phosphate absorption from the gut. High levels of vitamin D have been positively associated with bone geometry and bone mineral density in healthy older adults [139]. Children with CP display no differences in serum parathyroid hormone or vitamin D metabolism compared to

typically developing children [140-144] and no relationship between vitamin D and bone mineral density [143]. On the other hand, Tasdemir et al. [141] reported elevated serum calcium and phosphate in children with CP compared to typically developing children, but when the group with CP was separated by ambulatory status, serum calcium and phosphate were elevated only in the nonambulatory CP group.

Children with CP are at risk for stunted growth which is related to the level of motor impairment. Growth hormone (GH) and insulin-like growth factor (IGF1) are crucial for longitudinal bone growth [145]. The downstream effects of GH are mediated by GH-produced systemic and tissue-specific expression of IGF1. Systemic IGF1 has been shown to marginally modulate bone growth through a paracrine effect within the growth plate [146]. Results from Shufang et al. [147] indicate that ablation of the IGF1 receptor gene (*Igf1r*) in mice lead to stunted growth of the total body and tibia. When postnatal systemic GH was administered in *Igf1r* ablated mice, the stunted growth was blunted suggesting that GH mediates longitudinal bone growth at the growth plate independent of IGF1. The brain damage leading to CP may impact neurotransmitter pathways that control GH secretion [148] leading to GH deficiency [149] and poor GH responses to insulin administration [150] in children with CP. A study by Ali et al. [142] reported that children with CP had lower serum IGF1 and IGF1 binding protein z-scores which was more pronounced in nonambulatory vs. ambulatory children with CP [151]. Moreover, Hamza and colleagues [151] reported that GH, IGF1 and IGF1 binding protein negatively correlated with gross motor function ability and muscle spasticity in children with CP. Using high-resolution magnetic resonance imaging, Modlesky et al. [116] reported that trabecular bone microarchitecture immediately proximal to the femoral growth plate is underdeveloped by 30 % in nonambulatory

children with CP vs. typically developing study. In a later study, Modlesky et al. [115] reported that the CP participants had greater deficits of trabecular bone microarchitecture with distance from the growth plate in the metaphyseal region. While GH or systemic or local IGF1 was not measured in this study, it is plausible to think that the suppressed development of trabecular bone microarchitecture at the growth plate in children with CP was due, in part, to an altered GH-IGF1 axis.

Leptin is a hormone that is primarily secreted from fat cells. Leptin has both a central and peripheral effect on bone by innervating areas in the brain leading to sympathetic regulation of bone turnover. Whereas centrally-derived leptin drives sympathetic regulation that inhibits bone formation, localized expression of leptin in the bone cells promotes skeletal health by binding to the leptin receptors on the osteoblast and causing osteoblast proliferation, differentiation and secretion of mineralizing proteins from mature osteoblasts [152, 153]. Localized expression of leptin is correlated with bone area and changes in bone area in peripubertal women [154] and is an independent predictor of whole body and femoral neck BMD in postmenopausal women [155]. Continuous subcutaneous leptin administration helped to blunt the ovariectomized-induced trabecular bone loss, diminished trabecular architectural changes and low bone formation in ovariectomized rats [156]. Currently, not many studies have investigated leptin levels in children with CP. Tomoum et al. [157] reported no differences in serum leptin between children with CP and typically developing children. Yakut et al. [158] found that nonambulatory children with CP had lower serum levels of leptin compared to ambulatory children with CP. In a study of disabled vs. non-disabled children, the disabled children who had neurological impairments showed higher serum leptin levels compared to disabled children without

neurological impairments and girls had higher leptin levels than boys [159]. The few studies and mixed results conducted on leptin levels in children with CP and the lack of studies on its impact on bone metabolism in this population makes it difficult to draw conclusions on how this hormone influences skeletal status in children with CP. Furthermore, the predominance of leptin's central effect on bone may rely on the bone-marrow composition, leptin resistance at the hypothalamic level and the extent of neural innervation of bone [160, 161]; all factors that are currently unknown in the CP population.

### **2.3.3 Nerve-Bone Interaction**

A review by Turner and colleagues [162] proposed that bone cell characteristics resemble properties of neuronal systems in terms of responsiveness to stimuli. It has been suggested that bone cells have the ability of habituation, or cellular accommodation [163], therefore impacting bone cell responsiveness to mechanical or hormonal stimuli [164]. In a study of six healthy men aged 19 - 52 years undergoing seventeen weeks of bedrest, bone mineral loss was greatest in weight bearing bone sites (i.e., lower extremities, pelvis and lumbar spine) compared to non-weight bearing bone sites (i.e., ribs and upper extremities) with a redistribution of increased bone mineral to the skull [165]. Shear fluid pressure within the lacunar-canalliculi system of bone promotes bone cell adaptation. The fluid pressure is greater in the lower extremities vs. upper extremities when standing and even more so during ambulation and exercise. Rat ulnas subjected to external loading of 2 Hz for 360 cycles/day for 10 days found a varied strain magnitude along the diaphysis. The distal portion of the ulna diaphysis, compared to the middle and proximal portions, exhibited the greatest strain magnitude and also had the highest strain threshold to induce bone formation [166]. Taken together, these

data suggest that bone responds to the bone cell's perceived strain which varies depending on loading history and anatomical location. The process of bone cell learning and mechanosensitivity alterations in the short term are likely due to changes in neurotransmitter release [162] within the nerve-bone interface.

From the sensory neurons, glutamate is the neurotransmitter responsible for communication with motor neurons containing N-methyl-D-aspartic glutamate receptors. Habituation, or diminished responsiveness of cells to a stimulus, is due to decreased glutamate release from sensory neurons. Previous studies [167, 168] discovered neuronal glutamate/aspartate transporters and N-methyl-D-aspartic glutamate receptors in human osteoblastic cell lines and rat osteocytes, respectively. After exogenous loading bouts in rat ulnas, the expression of the bone tissue-derived glutamate/aspartate transporter was decreased suggesting a peripheral nerve-bone interaction in mechanically induced cellular habituation. Anatomical evidence of peripheral nerve-bone interactions is derived from an electron microscopy study showing glutamate-expressing nerve processes extending towards regions of trabecular bone and bone marrow along vessels in hematopoietic and bone cell rich environments [169].

Calcitonin gene-related peptide (CGRP) is a neurotransmitter that influences calcium metabolism and acts directly on osteoclasts to inhibit bone resorptive activity [170, 171]. Hara-Irie et al. [172] attempted to identify a relationship between CGRP-positive nerve fibers and bone cells in the epiphyseal and metaphyseal regions of the femur in growing rat pups. Using electron microscopy, the authors reported that CGRP-positive nerve fibers were densely populated in the growth plate and less densely populated in the metaphyseal region. Moreover, osteoclasts were regulated by CGRP-

positive nerve fibers which acted by inhibiting osteoclast maturation and activity and therefore bone resorption.

The sympathetic nervous system communicates through catecholamines, epinephrine and norepinephrine, which act as neurotransmitters. These catecholamines are released from the sympathetic nerves and the adrenal glands. Takeda et al. [173] surgically removed the adrenal medulla in wild type mice and four weeks later found no histological impact on bone mass. The authors concluded that this represented neuronal regulation of bone formation and thus, a central nerve-bone interaction. The study by Takeda et al. [173] also investigated central leptin and its associated anti-osteogenic function [174, 175] and sympathetic nervous system regulation of bone formation. When mice deficient in  $\beta$ -hydroxylase, an enzyme necessary for catecholamine production, were infused with leptin in the third ventricle, there was a loss of the gonadal fat pad, indicating body mass management which is one of the centrally-derived leptin actions. On the other hand, bone mass increased in the  $\beta$ -hydroxylase deficient mice compared to wild type, suggesting that leptin's anti-osteogenic action on bone requires an intact and functioning sympathetic nervous system. Central leptin exerts its anti-osteogenic effects by stimulating neuropeptide Y in the arcuate nucleus of the hypothalamus. This pathway encompasses the neuropeptide Y-associated leptin hypothalamic control of bone metabolism [174]. Baldock et al. [176, 177] showed that while central neuropeptide Y acts in response to central leptin signaling, central neuropeptide Y can regulate bone metabolism independent of leptin.

Taken together, neural regulation of bone is mediated by both central and peripheral commands. Children with CP have an initial disruption and gradual

heterogeneous under-maturation of the nervous system. Skeletal muscle tone is either abnormally higher (muscle spasticity) or lower (flaccid muscle) than typically developing children. It is therefore plausible to think that children with CP are more likely to have an altered sympathetic tone to the skeleton. The complications of this include disruptions of the hypothalamic control of bone metabolism upstream (brain) and downstream (periphery) and a less dense network of neural innervation of the growth plate that acts to inhibit bone resorption and promote bone formation. As previously mentioned, Modlesky et al. [116] reported lower trabecular bone microarchitecture in a 14 mm region immediately above the femoral growth plate in children with more severe forms of CP. When the pattern throughout the region was analyzed, the trabecular bone microarchitecture closest to the growth plate was 24 % lower while the trabecular bone microarchitecture furthest from the growth plate, in the metaphyseal region, was 34 % lower compared to typically developing children [115], suggesting a more pronounced deficit of trabecular bone microarchitecture with distance from the growth plate. In the study by Hara-Irie et al. [172], a neural network that directly inhibits bone resorption was found to be more dense in the growth plate and less dense in the metaphyseal region in growing rat pups. The reason for the greater discrepancy of lower trabecular bone microarchitecture further from the growth plate, and in the metaphyseal region, in children with CP could be speculated to be a result of a disproportionate deficit in neural innervation from the epiphyseal to metaphyseal region, a lack of physical activity and therefore reduced mechanical stimulus, altered sympathetic tone or a unique combination of these factors.

### 2.3.4 Bone Marrow Fat

Seminal studies dating back to the 1970s have demonstrated an inverse relationship between trabecular bone and bone marrow fat in humans and animals [43, 44, 178]. This inverse relationship is consistent in the diaphysis, where cortical bone is predominant, in healthy young [179-181] to older [180] adults. Bone marrow-derived mesenchymal progenitor cells have a multi-potency to differentiate into an array of musculoskeletal lineages [182]. Particularly, within the bone marrow cavity, these progenitors have a bi-potential differentiation capacity of either an osteogenic or adipogenic (i.e., fat) program [183]; thereby acting as a key regulator of skeletal homeostasis. Although fat cell filling of the bone marrow cavity was once considered passive and inert [43, 44, 178, 184], it is now recognized that fat tissue has autocrine, paracrine and classic endocrine effects. Bone marrow fat is a relatively new area of research and little is known about its metabolic and endocrine effects on local and distant organs. However, it has been documented that high levels of bone marrow fat are associated with markers of poor bone health [185-187]. In those with skeletal disease, the lineage commitment of mesenchymal progenitor cells may not be as simple as a switch from an osteogenic to an adipogenic program [41, 42] as is suggested in healthy adults [41, 179-181]. Using micro-computed tomography and bone histomorphometry of iliac bone biopsies, Cohen et al. [41] reported that premenopausal women with skeletal disease had significantly lower trabecular bone microarchitecture compared to controls ( $p < 0.05$ ). The skeletal disease group also had higher fat cell number, size and volume compared to controls (all,  $p < 0.05$ ). The relationships between fat cell volume, trabecular bone microarchitecture and bone formation rate were reciprocal in the controls ( $r = -0.41$  to  $-0.64$ , all,  $p < 0.05$ ) but no significant relationships were observed in the skeletal disease group ( $r = -0.03$  to  $-0.12$ , all,  $p > 0.3$ ).

Verma et al. [188] reported similar findings in human osteoporotic iliac crest bone biopsies from men and women.

In healthy adults, increases in bone marrow fat would be due to a shift in the mesenchymal progenitor cell metabolism favoring adipogenesis resulting in a greater number of bone marrow fat cells at the expense of osteoblasts. Therefore, one unit increase in bone marrow fat would coincide with a proportionate unit decrease in bone tissue, which was observed in the controls from the study by Cohen et al. [41]. On the other hand, in adults with skeletal disease, increases in bone marrow fat would be due to a shift in the mesenchymal progenitor metabolism favoring adipogenesis while the bone marrow tissue may be additionally promoting fat cell enlargement. Therefore, adults with skeletal disease may exhibit a disproportionate increase in bone marrow fat tissue, relative to the bone tissue deficit, due to increases in the number (mesenchymal progenitor cell metabolism favoring adipogenesis) and volume (post-differentiation modifications) of bone marrow fat cells. The idea of post-differentiation modifications occurring in the mature bone marrow fat cells causing cellular enlargement may be masking the relationship between bone and bone marrow fat in adults with skeletal disease, such as in the study by Cohen et al. [41].

Bone marrow tissue is highly complex and composed of many different cellular lineages. In addition to bone and fat tissue, bone marrow also contains hematopoietic tissue. Verma et al. [188] reported a greater proportion of bone marrow fat relative to hematopoietic tissue from iliac crest biopsies from osteoporotic men and women. In accordance, Justesen et al. [189] demonstrated that iliac crest biopsies from osteoporotic men and women exhibit a greater proportion of bone marrow fat volume and less bone and hematopoietic volume relative to total bone marrow volume. This suggests that

bone marrow fat can accumulate at the expense of bone and hematopoietic tissue. This latter notion is supported by an excellent publication in Nature by Naveiras et al. [190] that reported on a series of experiments on mice in two vertebral bone marrow locations; the thoracic vertebrae, which contains active hematopoietic tissue and is devoid of fat, and the tail vertebrae, which contains active hematopoietic and fat tissue. The following results are reported based on the comparison between the two different vertebral bone marrow sites (i.e., thoracic and tail) and are as follows: 1) bone marrow fat cells reduce the expansion of hematopoietic cells, 2) bone marrow fat cells functionally reduce the cell cycle of many bone marrow-derived stromal cell compartments and 3) there is a greater proportion of quiescent stem cells in regions of enriched bone marrow fat, indicating a functional inhibition in the differentiation of many bone marrow-derived progenitor cells. Furthermore, when they compared wild type mice with mice genetically devoid of bone marrow fat in all vertebrae, the genetically modified mice had a faster hematopoietic recovery rate following total body irradiation of the bone marrow.

Taken together, bone marrow fat accumulates at the expense of bone and hematopoietic tissue and may have a direct role on bone and hematopoietic function. Considering that bone marrow fat research is a relatively new and emerging field of scientific and clinical investigation, there are many questions that need to be addressed. For example, does bone marrow fat accumulate similarly or differently in healthy vs. diseased states? What is the metabolic function of bone marrow fat cells in disease and is it dependent on the origin of disease (e.g., genetic alterations, neurological lesions, physical inactivity, insulin resistance, cancer, etc.)? What is the metabolic function of bone marrow fat cells during different stages of growth and development? Does bone

marrow fat accumulate or functionally differ preceding, following or in parallel with bone loss? These questions are vital in understanding the pathogenesis of bone and other diseased states, especially in times of growth and development and is of particular importance to those with CP given the musculoskeletal deficits observed.

To date, the fat profile of bone marrow in children with CP is unknown. However, by the age of 10 years, children with more severe forms of CP have a marked deficit in total, cortical and medullary volume of the femur, up to 48 % thinner cortical walls and a devastating 66 % bone strength deficit when compared to typically developing children [34]. Whether these architectural and strength deficits are associated with an increased accumulation of bone marrow fat is unknown. Bone marrow fat was shown to be inversely related to computed tomography-derived femoral cortical bone area in healthy adolescent women and was also an independent predictor of changes in bone geometry after 18 months in this group [179]. Rantalainen et al. [191] reported similar results after conducting a cross-sectional study in young premenopausal athletes who had a 10 year competitive athletic history of either impact sports (e.g., soccer, jumpers, power lifters, etc.) or a nonimpact sport (i.e., swimming) and controls. However, in this study, the authors found that the impact group showed greater bone geometry measures and less bone marrow fat compared to the other two groups (i.e., nonimpact group and controls) while no group differences in bone geometry or bone marrow fat were observed between the nonimpact group and controls. These data suggest that while physical activity is important for bone development, the type of physical activity, and therefore mechanical loading regime, is also important for bone health.

Maintenance of healthy bone turnover during skeletal modeling and remodeling is regulated by bone-forming osteoblasts and bone-resorbing osteoclasts. These cells have the ability to regulate the other cell in various stages of cellular development via direct and indirect regulatory factors [192-194]. In regards to osteoblast regulation of osteoclasts, one crucial pathway involves the early and late osteoblast expression and release of receptor activator of NfκB ligand (RANKL) and osteoprotegerin (OPG), respectively. By competitive binding to the same surface RANK receptors of osteoclast progenitors, RANKL induces formation and maturation of osteoclasts whereas OPG inhibits these actions.

In the bone marrow cavity, hematopoietic stem cells differentiate towards osteoclasts while mesenchymal progenitor cells differentiate into osteoblasts or bone marrow fat cells. The inverse relationship and regulation between bone marrow fat and bone has been well established [43, 44, 178]. More recent evidence shows the potential for human bone marrow fat cells in regulating osteoclastogenesis via osteoclast regulatory molecules [195, 196]. Holt et al. [197] recently reported in vitro analysis of mesenchymal progenitor cell-derived bone marrow fat cells expressing RANKL and OPG that were capable of supporting osteoclast-like cell formation. These studies [195-197] provide evidence that bone marrow fat cells have a biological role on both osteoblasts and osteoclasts, thereby acting as an important regulator of bone turnover. The regulatory mechanism of bone marrow fat cells on osteoclasts may be explained, in part, by inflammation.

Inflammatory cytokines, such as interleukin (IL)-1 $\beta$ , IL-6 and tumor necrosis factor (TNF)- $\alpha$ , are abundant in adipose tissue and are associated with metabolic dysregulation [198-200], osteoclastogenesis and bone resorption by acting through the

RANKL/RANK/OPG pathway [201, 202]. Halade et al. [203] reported that mice that were fed a high fat diet exhibited a markedly reduced trabecular bone microarchitecture, greater number and size of bone marrow fat cells, higher RANKL expression and lower OPG expression from the femur. These mice also had an increased gene expression of inflammatory cytokines such as IL-1 $\beta$ , IL-6 and TNF- $\alpha$  and the upregulation of PPAR $\gamma$  (a primary adiogenic transcription factor) and downregulation of runx2 (a primary osteogenic transcription factor).

Children with CP have a poor accretion of bone mass leaving them vulnerable to low energy fractures. To date, bone marrow fat characteristics has not been evaluated in those with CP. This information is needed to better understand how bone marrow fat influences bone metabolism during growth, bone material properties, localized inflammation and systemic energy metabolism, such as factors relating to cardiometabolic disease.

#### **2.4 Cardiometabolic Disease Risk**

Adults with CP have a two to three times greater risk of cardiovascular mortality [204] and are predisposed to a wide array of cardiometabolic diseases due to low levels of physical activity and blunted musculoskeletal growth [118, 119, 205]. In a study published in 2014, the prevalence of metabolic syndrome was higher in nonambulatory adults with CP (29 %) [205] than that previously reported for the general population of American adults aged 20 years and older from 2009 and 2010 (23 %) with data coming from the National Health and Nutrition Examination Survey [206]. In the largest CP study to date (n = 435), Cremer, Hurvitz and Peterson [207] reported a high prevalence of multimorbidity (58 %) in middle-aged adults with CP. There were 137 unique combinations of multimorbidity in the whole cohort with the 3 most prevalent

combinations including cardiovascular and musculoskeletal and musculoskeletal diseases. Moreover, obesity was an independent predictor of multimorbidity prevalence suggesting that musculoskeletal and cardiometabolic systems are highly integrated in terms of chronic disease progression in those with CP.

#### **2.4.1 Body Composition and Cardiometabolic Disease Risk in CP**

Children with cerebral palsy (CP) are at an increased risk for having a misclassified overweight/obesity status based on body mass index (BMI) and percent body fat (%Fat), commonly used methods to assess body composition in children, because of their lower fat-free mass [35, 208, 209], but similar fat mass [35, 210, 211] compared to typically developing children. BMI-based thresholds suggest the prevalence of obesity in children with mild-to-moderate CP [212] is similar to [213] or slightly lower than [214] the general population of children and that ambulatory children with CP have a greater likelihood of being overweight/obese than children with CP with greater levels of motor impairment [212, 215]. It is possible that these findings are driven by the greater musculoskeletal deficits seen in nonambulatory than ambulatory children with CP [18, 34, 216] rather than lower accretion of fat. Therefore, BMI may not be capturing the true level of body fat and leanness in children with CP, which may be further complicated by ambulatory status.

A study of 31 adults with CP compared to 311 matched controls reported that the CP group had higher visceral but not subcutaneous abdominal fat. However, after adjusting for age, sex and body mass, visceral fat remained higher and subcutaneous emerged as higher in CP compared to controls [217]. How obesity indices impact cardiometabolic disease risk in children with CP is unknown. Moreover, if a particular

obesity index (e.g., total body fat, intramuscular fat, visceral fat, etc.) is more related to cardiometabolic disease in children with CP is also unknown.

#### **2.4.1.1 Cardiometabolic Disease Risk Factors**

Abnormal glucose and insulin regulation predisposes cardiometabolic diseases [218]. In a meta-analysis of 65 studies and over 500,00 adult participants, insulin resistance was associated with cardiovascular disease and all-cause mortality [219]. Kurl et al. [220] followed over 2,500 nondiabetic men for twenty years to determine the association between coronary heart disease mortality and insulin resistance and fasting serum insulin. Using multivariable Cox regression analysis adjusting for age, BMI, systolic blood pressure, cholesterol, cigarette smoking, history of coronary heart disease, alcohol consumption, blood leukocytes and plasma fibrinogen, the reported hazard ratios for coronary heart disease mortality were 1.69 ( $p = 0.008$ ) for insulin resistance and 1.59 ( $p = 0.016$ ) for fasting serum insulin when comparing top vs. bottom quartiles. The fasting serum glucose had a nonsignificant hazard ratio of 1.26 ( $p = 0.173$ ). However, 96 % of the subjects had baseline fasting serum glucose levels (group average =  $4.6 \pm 0.5$  mmol/L) lower than the threshold reported for cardiovascular disease risk (5.6 mmol/L), therefore explaining the potential lack of association [220].

#### **2.4.1.2 Obesity Indices and Cardiometabolic Disease Risk**

Obesity is associated with a wide array of health complications. Obesity in childhood is associated with increased morbidity and mortality of patients with cardiometabolic diseases in adulthood [221, 222]. In a study of normoglycemic overweight and obese children, fasting plasma insulin and glucose and insulin response to glucose loading were correlated with a number of cardiovascular disease risk factors

[223]. Burke et al. [224] further demonstrated that fasting insulin and glucose levels are positively related to measures of obesity and cardiometabolic disease risk factors in children. Kovanlikaya and colleagues [39] demonstrated that non-diabetic adolescent obese females had greater fasting insulin levels compared to their leaner counterparts. Furthermore, when the groups were combined, there was a positive relationship between fasting serum insulin and total body fat ( $r^2 = 0.61, p < 0.001$ ). Total body fat is the sum of fat from various fat depots. Obesity is associated with excess fat stored in the subcutaneous, visceral and liver depots [225]. This is a concern because not all fat depots are created equal in terms of cardiometabolic disease risk and long term glucose dysregulation leads to insulin resistance and T2DM.

Adolescents and adults with T2DM have elevated levels of total body, subcutaneous, visceral, liver and pancreas fat compared to the general population [39, 225, 226]. High infiltration of fat in the liver is of concern because the liver is the second major storage site for glucose, second to muscle, and would therefore impede with glucose uptake leaving a high concentration of glucose in the blood. High infiltration of fat in the pancreas is of concern because the pancreas releases insulin to lower blood glucose and glucagon to elevate blood glucose. A disruption in the endocrine control mechanism of blood glucose from the pancreas leads to metabolic abnormalities, such as insulin resistance if insulin secretion is impaired. Indeed, higher fat infiltration of the liver and pancreas is associated with elevated levels of fasting serum insulin [39].

Abdominal fat distribution may also play a critical role in insulin resistance. A study conducted on men and women with T2DM found significant correlations between peripheral and hepatic insulin resistance and adiposity indices, such as total body,

visceral and subcutaneous fat (all,  $r^2 > 0.14$ ,  $p < 0.05$ ). Kelly et al. [227] investigated the association of abdominal visceral and subcutaneous fat with cardiometabolic disease risk factors in typically developing children. Using linear regression analysis adjusted for age, sex, race, sexual maturation and total body fat, visceral and subcutaneous fat were independent predictors for markers of insulin resistance and other cardiometabolic disease risk factors (all,  $p < 0.05$ ). Importantly, the adjusted relationships between visceral and subcutaneous fat and cardiometabolic disease risk factors were stronger in children with visceral and subcutaneous fat at or above the mean for the whole group. This suggests a threshold for fat accumulation in the visceral and subcutaneous depots that is related to a nonlinear increase in cardiometabolic disease risk. However, not all studies have found such a strong association of subcutaneous fat and cardiometabolic disease risk. Some studies have found that visceral fat is a stronger predictor of glucose metabolism than subcutaneous fat [228] and that the development of pre-diabetes and diabetes is related to visceral fat and not subcutaneous fat [229] in the abdominal region. Moreover, Goodpaster et al. [38] reported a lack of association between regional thigh subcutaneous fat and insulin sensitivity.

The difference of the role on cardiometabolic disease risk between visceral and subcutaneous fat may have to do with the anatomical distribution of the fat depots in relation to key organs involved in glucose regulation. Visceral fat is located around abdominal organs, such as the liver and pancreas, and would be more likely to interfere with normal physiological processes than subcutaneous fat when elevated leading to central-derived insulin resistance. However, excess free fatty acids inhibit skeletal muscle glucose uptake and since visceral fat contributes only 15 % of the total systemic

free fatty acid pool [230, 231], the role of visceral fat on peripheral (muscle) insulin sensitivity has been doubted [232].

#### **2.4.2 Intramuscular Fat and Cardiometabolic Disease Risk**

Muscle tissue is the main storage and utilization site for glucose. Elevated fat within muscle interferes with glucose uptake and storage leading to elevated blood glucose and insulin [39]. If left unchecked, elevated blood glucose will lead to irregular glucose handling at the level of the myocyte, peripheral insulin resistance and followed by central-derived insulin resistance in response to damage to the  $\beta$  cells that produce and secrete insulin. Although skeletal muscle fat accounts for a small fraction of total fat tissue at the same cross-section, skeletal muscle fat depots have a greater role in peripheral insulin resistance than other fat depots (e.g., subcutaneous and total) in the periphery [38].

##### **2.4.2.1 Intramyocellular Lipid**

Skeletal myocytes sequester plasma free fatty acids where up to 90 % are partitioned to either mitochondrial oxidation for fuel metabolism or, in times of low energy demand, partitioned for storage in intramyocellular lipid (IMCL) [233]. Competitive athletes and obese individuals have elevated intramyocellular TG content. The elevated intramyocellular TG content in athletes is part of an upregulation of energy supply due to a greater energetic capacity coinciding with a high rate of intramyocellular TG turnover and content [95-97]. On the other hand, in obesity, there is a high level of plasma free fatty acids [234]. This results in an oversupply of intramyocellular fatty acids which is associated with mitochondrial dysfunction [235]. The excessive accumulation of intramyocellular TG results in increased IMCL storage. The

combination of mitochondrial dysfunction and increasing IMCL formation creates an intracellular lipotoxic environment. Lipid toxicity is orchestrated by the excessive production of lipid intermediates by IMCL that are thought to evoke intramyocellular oxidative stress leading to myocyte insulin resistance [40, 236].

Glucose uptake into cells is mediated by insulin and the most abundant myocellular glucose transporter is GLUT4. In a T2DM-induced mouse model, Alam et al. [237] reported elevated fasting serum glucose, skeletal muscle and liver oxidative stress, impaired glucose utilization and lower skeletal muscle-derived GLUT4. Facilitation of fatty acid uptake into myocytes is initiated by insulin-mediated translocation of CD36 from intramyocellular vesicles to the membrane of the sarcolemma [233]. There is evidence in adults with insulin resistance suggesting a greater degree of fatty acid transport into the myocytes creating greater intramuscular TG content in response to a more permanent translocation of CD36 [238]. Increased insulin-mediated translocation of CD36 may initiate or exacerbate the pathways leading to lipotoxicity and inadvertently prevent cytosol-derived GLUT4 translocation to the cellular membrane that allows for myocellular glucose uptake. Although no studies have directly investigated this idea of lipotoxicity disrupting GLUT4 activity, it is plausible that herein lies the fundamental element of muscle-derived insulin resistance. However, other factors to consider is how the liver and pancreas interact with skeletal myocyte glucose-insulin axis impairment.

#### **2.4.2.2 Extramyocellular Lipid**

To date, no studies have investigated a direct biochemical or physiological role of extramyocellular lipid (EMCL) on glucose regulation. However, EMCL resides in adipocytes located between muscle fibers and muscle bundles. If elevated or

metabolically altered, EMCL may play a role at the binding site of insulin and its cell surface receptor and may impede insulin-mediated glucose uptake from outside the cell. Although, this is only speculation at this point. Future studies are required.

### **2.4.3 Bone Marrow Fat and Cardiometabolic Disease Risk**

The main finding from a study by Huovinen and colleagues [45] follows that vertebral bone marrow fat content is inversely related to vertebral bone marrow glucose metabolism in a healthy and diabetic animal model. It has been shown that fat tissue generally has a low and heterogeneous glucose uptake depending on the anatomical site investigated [239]. Given that bone marrow fat accumulates at the expense of bone and hematopoietic tissue [188, 189], which both use glucose as a substrate for metabolism, the inverse relationship between bone marrow fat and impaired glucose metabolism may be in response to a direct effect of the increased bone marrow fat tissue or an indirect effect of the replacement of bone and hematopoietic tissue within the bone marrow cavity.

In a recent study, magnetic resonance imaging revealed that while vertebral bone marrow fat in adults is ~54 % and femur/tibia bone marrow fat is ~85 %, the axial and appendicular bone marrow fat sites were correlated ( $r = 0.361$  to  $0.780$ , all,  $p < 0.05$ ). The authors also reported positive relationships between bone marrow fat of the axial and appendicular sites with cardiometabolic disease risk factors [240]. Baum et al. [46] demonstrated that postmenopausal T2DM women have a positive relationship between vertebral bone marrow fat and glycated hemoglobin (a more stable measure of long term glucose load), but no such relationship was observed in healthy postmenopausal women. When they split the T2DM women into two groups that either fell below or above 7 % glycated hemoglobin levels, a target value that improves microvascular function in

adults with T2DM [47], the above group had greater vertebral bone marrow fat than the below group. Taken together, bone marrow fat disrupts glucose uptake and utilization directly or indirectly at the local level and is associated with systemic dysregulation of cardiometabolic disease risk factors.

## **2.5 Conclusions**

Children with CP have developmental challenges due to the original neurological insult of the developing brain leading to neuromuscular dysfunction, low levels of physical activity and inadequate accretion of muscle and bone. To give a sense of urgency, each one of these factors is independently associated with increased risk for cardiometabolic diseases. Therefore, a multitude of these factors is of extreme clinical concern. Inferences from reliable clinical populations suggest that those with CP are more prone to altered fat distribution of the abdomen and elevated fat infiltration of the musculoskeletal system, which acts, in part, to exacerbate their childhood-onset complications and may act as a platform to initiate or exacerbate frailty and chronic disease progression. Therefore, there is a need to identify factors associated with cardiometabolic disease risk, such as musculoskeletal, abdominal and total body fat, in children with spastic CP.

## **2.6 Specific aims**

My long term goal is to mitigate chronic disease risk in individuals with CP. The overall objective of my study was to examine the local and global fat profile in children with CP and to determine how fat distribution relates to muscle strength and physical activity. My central hypothesis was that, when compared to typically developing children, children with spastic CP will have higher fat within the leg

musculoskeletal system which will be negatively related to muscle strength. Further, compared to typically developing children, children with spastic CP will have higher abdominal and total body fat. The rationale for my study was that it will allow us to better understand musculoskeletal pathophysiology and factors relating to cardiometabolic disease risk in children with spastic CP.

**Specific Aim 1.** To determine if ambulatory children with spastic CP have a different fat distribution profile of the leg compared to typically developing children.

Hypothesis 1.1: Compared to typically developing children, ambulatory children with spastic CP will have no difference in total or subcutaneous fat of the leg.

Hypothesis 1.2: Compared to typically developing children, ambulatory children with spastic CP will have higher subfascial, intermuscular, intramuscular and bone marrow fat of the leg.

**Specific Aim 2.** To determine the volume and fat concentration profile of individual leg muscles and how it relates to muscle strength in ambulatory children with spastic CP.

Hypothesis 2.1: Compared to typically developing children, ambulatory children with spastic CP will have lower muscle volume and higher fat concentration of all individual leg muscles.

Hypothesis 2.2: Compared to typically developing children, ambulatory children with spastic CP will have lower ankle strength per unit of muscle. Intramuscular fat will help to partially explain the difference in muscle strength between groups.

**Specific Aim 3.** To determine if ambulatory children with spastic CP have higher abdominal fat compared to typically developing children.

Hypothesis 3.1: Compared to typically developing children, ambulatory children with spastic CP will have higher abdominal visceral and subcutaneous fat.

**Specific Aim 4.** To determine if body mass index (BMI) can accurately estimate body composition in children with CP.

Hypothesis 4.1: BMI would correlate fat mass index (FMI) and fat-free mass index (FFMI) but would underestimate FMI and overestimate FFMI in children with CP and these discrepancies would be more pronounced in nonambulatory than ambulatory children with CP.

Findings from the proposed study will lead to not only a better understanding of ectopic fat accretion within the musculoskeletal system and how it relates to muscle function, but will also lead to future rehabilitation strategies focused on the muscles that are most severely impaired and mitigating fat accumulation in deleterious depots.

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## Chapter 3

### **CORTICAL BONE DEFICIT AND FAT INFILTRATION OF BONE MARROW AND SKELETAL MUSCLE IN AMBULATORY CHILDREN WITH MILD SPASTIC CEREBRAL PALSY**

#### **3.1 Abstract**

**INTRODUCTION:** Nonambulatory children with severe cerebral palsy (CP) have underdeveloped bone architecture, low bone strength and a high degree of fat infiltration in the lower extremity musculature. The present study aims to determine if such a profile exists in ambulatory children with mild CP and if excess fat infiltration extends into the bone marrow.

**MATERIALS AND METHODS:** Ambulatory children with mild spastic CP and typically developing children (4 to 11years; 12/group) were compared. Magnetic resonance imaging was used to estimate cortical bone, bone marrow and total bone volume and width, bone strength [i.e., section modulus ( $Z$ ) and polar moment of inertia ( $J$ )], and bone marrow fat concentration in the midtibia, and muscle volume, intermuscular, subfascial, and subcutaneous adipose tissue (AT) volume and intramuscular fat concentration in the midleg. Accelerometer-based activity monitors worn on the ankle were used to assess physical activity.

**RESULTS:** There were no group differences in age, height, body mass, body mass percentile, BMI, BMI percentile or tibia length, but children with CP had lower height percentile (19<sup>th</sup> vs. 50<sup>th</sup> percentile) and total physical activity counts (44%) than controls (both  $p < 0.05$ ). Children with CP also had lower cortical bone volume (30%), cortical

bone width in the posterior (16%) and medial (32%) portions of the shaft, total bone width in the medial-lateral direction (15%), Z in the medial-lateral direction (34%), J (39%) and muscle volume (39%), and higher bone marrow fat concentration ( $82.1\pm 1.8\%$  vs.  $80.5\pm 1.9\%$ ), subfascial AT volume (3.3 fold) and intramuscular fat concentration ( $25.0\pm 8.0\%$  vs.  $16.1\pm 3.3\%$ ) than controls (all  $p<0.05$ ). When tibia length was statistically controlled, all group differences in bone architecture, bone strength, muscle volume and fat infiltration estimates, except posterior cortical bone width, were still present (all  $p<0.05$ ). Furthermore, a higher intermuscular AT volume in children with CP compared to controls emerged ( $p<0.05$ ).

**CONCLUSIONS:** Ambulatory children with mild spastic CP exhibit an underdeveloped bone architecture and low bone strength in the midtibia and a greater infiltration of fat in the bone marrow and surrounding musculature compared to typically developing children. Whether the deficit in the musculoskeletal system of children with CP is associated with higher chronic disease risk and whether the deficit can be mitigated requires further investigation.

### **3.2 Introduction**

Cerebral palsy (CP) is a neurological condition that is associated with dysfunctional gait and progressive decrements in physical activity from childhood to adulthood [1]. Nonambulatory children with more severe spastic CP present with low bone mass and underdeveloped bone architecture [2-6], as well as small, weak [7] and qualitatively-compromised musculature, as indicated by a high degree of fat infiltration [8]. It is not surprising that this musculoskeletal phenotype is associated with a high incidence of low-energy fractures, primarily occurring in the lower limbs [9, 10]. The latter complication may be due mainly to the lack of physical activity [4, 8] and

mechanical loading, which reduces the stimulus for periosteal and endocortical expansion [4] in the lower extremities. To date, it is unclear if the adverse musculoskeletal profile exhibited in nonambulatory children with more severe CP is also present in ambulatory children with a milder form of the disorder.

In addition to possessing an underdeveloped musculoskeletal system, nonambulatory children with severe CP also have elevated adipose tissue (AT) surrounding bone and muscle [8]. Furthermore, human models of reduced mechanical loading show elevated levels of fat infiltration within the bone marrow cavity [11] and muscle [12], which are linked to osteoporosis [13-15], impaired glucose tolerance [12, 16, 17] and cardiometabolic disease [18]. The objective of the present study was to determine if ambulatory children with mild spastic CP have a deficit in bone architecture and elevated fat infiltration within the bone marrow cavity of the midtibia and the surrounding leg musculature. We hypothesized that ambulatory children with mild CP vs. typically developing children would have a thinner shaft with a thinner cortex and lower estimates of strength in the midtibia. We also hypothesized that children with CP vs. typically developing children would have an elevated fat infiltration within the bone marrow cavity and the surrounding leg musculature.

### **3.3 Materials and Methods**

#### **3.3.1** *Participants and Study Design*

Ambulatory children with mild spastic CP and between the ages of 4 and 11 y were recruited from the AI duPont Hospital for Children in Wilmington, DE and other pediatric hospitals in the Mid-Atlantic region of the U.S. Typically developing children that matched children with CP for age, sex and race were recruited from the Newark

and Wilmington, DE areas using flyers and word of mouth. Additional inclusion criteria for controls included falling between the 5<sup>th</sup> and 95<sup>th</sup> percentile for height and body mass, no history of chronic medication use, no previous fracture in the nondominant lower extremity and no current or previous regular participation in an activity that involved high loading of the skeleton, such as artistic gymnastics. Participants were recruited from April 2012 through May 2016 and testing was conducted from November 2012 to May 2016. The Institutional Review Boards at the AI duPont Hospital for Children and the University of Delaware approved the study procedures. Prior to testing, written consent and assent was obtained by the parents and the participants, respectively.

### **3.3.2** *Anthropometrics*

Height and body mass were measured while the child was in a t-shirt and shorts. Height was measured to the nearest 0.1 cm using a stadiometer (Seca 217; Seca GmbH & Co. KG., Hamburg, GER). Body mass was measured to the nearest 0.2 kg using a digital scale (Detecto 6550, Cardinal Scale, Webb City, MO). Height, body mass and BMI percentile were calculated from the normative graphs published by the Centers for Disease Control and Prevention [19].

### **3.3.3** *Gross Motor Function*

Gross motor function of children with CP was assessed by a physician assistant using the gross motor function classification system (GMFCS) [21]. Children who were GMFCS I or II were included in the study. In short, a child with the ability of walking indoors and outdoors and gross motor skills of running and jumping, but limited ability of speed, balance and coordination was classified as GMFCS level I. Limitations of

walking on uneven surfaces and inclines and minimal gross motor skills of running and jumping was classified as GMFCS level II.

### **3.3.4** *Physical Activity*

Physical activity was estimated using accelerometer-based activity monitors (Actical; Respironics Inc., Bend, OR). The activity monitors contain an omnidirectional accelerometer that is most sensitive to movements in the vertical plane when worn on the ankle and is sensitive to movements in the 0.5 to 3 Hz frequency range [22]. Physical activity counts were registered in 15 second epochs. Each participant wore two monitors on the lateral aspect of the ankle on the more affected side in children with CP and on the nondominant side in controls. Monitors were worn continuously (i.e., 24 hours per day) for four days (three week days and one weekend day). Participants and participant parents were instructed to take the monitors off only when swimming at a depth greater than 0.91 meters and during bathing/showering. This was confirmed by reviewing activity logs kept by the children with assistance from their parent and by visually examining the graphical output generated using software provided by the manufacturer. If participants did not wear the monitors on any of the days, they were asked to re-wear the monitors to make up for the lost day(s). The total physical activity counts per day averaged from the two monitors are reported. The reliability of the total physical activity counts was assessed in 8 ambulatory children with mild CP and 8 typically developing children between 4 and 11 years of age who wore the monitors for four days on two separate occasions approximately one month apart. The intraclass correlation was 0.935 for children with CP and 0.913 for typically developing children indicating excellent reliability.

### 3.3.5 *Magnetic Resonance Imaging*

Magnetic resonance imaging (MRI; GE, 1.5 T, Milwaukee, WI) was used to assess bone architecture and the degree of fat infiltration in the bone marrow at the level of the middle-third of the tibia (i.e., midtibia) and in the surrounding leg musculature (i.e., midleg). The more affected limb in children with CP and in the nondominant limb in controls were tested. Children were immobilized from the waist down using the BodyFIX (Medical Intelligence, Inc., Schwabmünchen, GER), as previously described [3]. A three plane localizer was used to identify the region of interest. Axial images were collected from the tibia plateau to the malleolar articular surface (0.5 cm thick separated by 0.5 cm of spacing) using a semiflex long bone array coil (ScanMed, Omaha, NE) and two different sequences. The first sequence (fast spin echo, TR = 650, TE = 14, FOV = 12, NEX = 3, BW = 15.63, frequency = 512, phase = 256) yielded T1-weighted images. The second sequence (IDEAL: fast-spin-echo, TR = 600, TE = min full, FOV = 12, NEX = 2, BW = 31.25, frequency = 320, phase = 224) yielded fat and water images.

All image collection was overseen by the senior author (CMM). Images at the level of the midtibia were processed blindly by the same technician using software developed with Interactive Data Language (Research Systems, Inc, Boulder, CO) and procedures previously described for the mid thigh [8]. A general visual description of the image processing procedure used to quantify bone architecture and strength of the midtibia and the fat infiltration of the bone marrow and surrounding musculature is provided in **Figure 1**. A more specific description follows. Using an automated procedure, the T1-weighted images at the level of the middle-third of the tibia were filtered using a median filter and voxels were segmented and assigned to cortical bone, bone marrow, muscle and AT using a fuzzy clustering algorithm [23] and their volumes

(cm<sup>3</sup>) were calculated. Widths (cm) of the cortical bone in the anterior, posterior, medial and lateral portions, widths of the medullary cavity and total bone in the anterior-posterior and medial-lateral directions and estimates of bone strength [i.e., polar moment of inertia (J) and section modulus] were also calculated during the same procedure. As described previously [4], the parallel-axis theorem [24] was used to determine the cross-sectional moment of inertia of the midtibia in the anterior-posterior and medial-lateral directions. Polar moment of inertia (cm<sup>4</sup>) was calculated by averaging the cross-sectional moment of inertia in the two directions. Section modulus (Z; cm<sup>3</sup>) was calculated by dividing cross-sectional moment of inertia measure by the furthest distance from the neutral axis in the anterior-posterior (Zap) and medial-lateral (Zml) directions. Tibia length was estimated by counting the number of images used to cover the entire midtibia and adjusting for image thickness and spacing. The test-retest reliability for measures of bone architecture, bone strength and muscle size measures in the lower extremity from MRI was assessed previously in a combined sample of children with CP and typically developing children tested twice on separate days or on the same day after repositioning [4, 8]. Intraclass correlation coefficients > 0.99 and coefficients of variation between 0.6 and 2.6 % indicated excellent reliability.

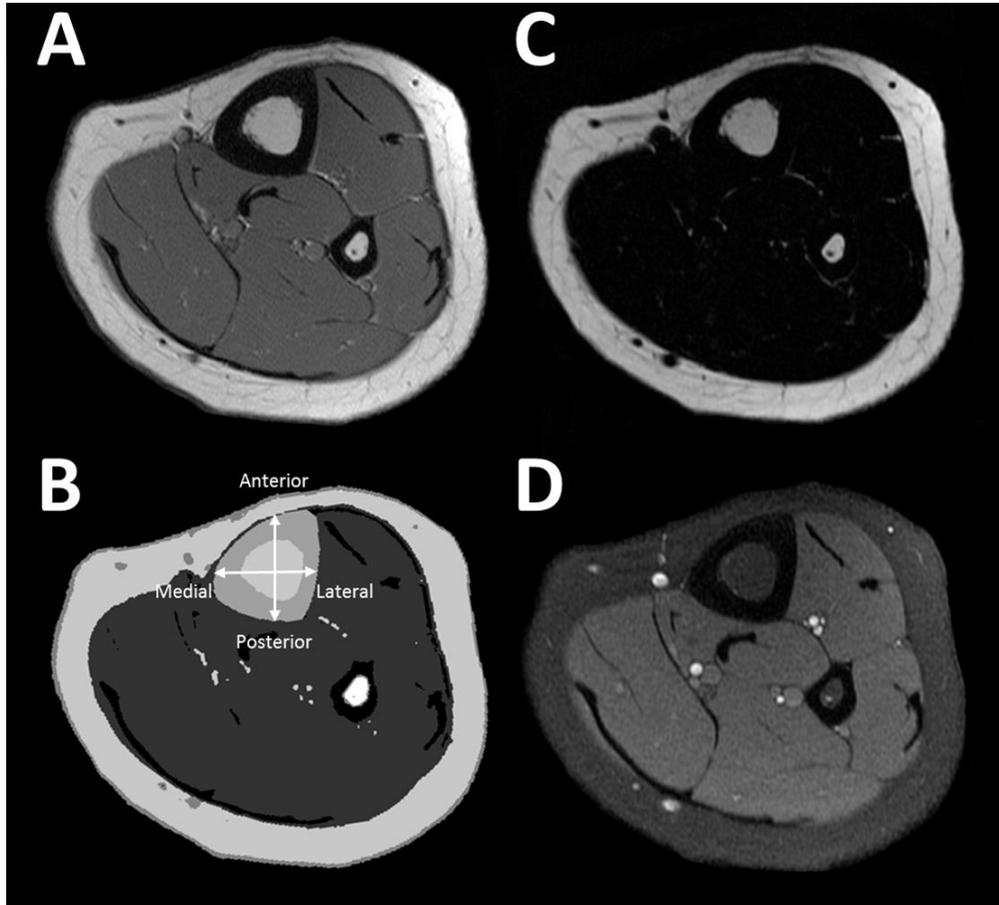


Figure 3.1. A general description of the image processing procedure used to quantify bone architecture and strength of the midtibia and the fat infiltration of the bone marrow and surrounding musculature. Raw T1-weighted magnetic resonance images (A) depict tibia cortical bone (large black ring; fibula is the small black ring) and bone marrow (white region surrounded by the cortical bone), muscle (gray region surrounding the bone) and adipose tissue (AT; white ring surrounding the muscles and bones and the white voxels interspersed among the musculature) which were segmented (B) and assigned to their tissue-specific regions. The bone, muscle and AT volumes, bone widths (cortical bone in the anterior, posterior, medial and lateral portions and the bone marrow and total bone in the anterior-posterior and medial-lateral directions) and estimates of bone strength (section modulus in the anterior-posterior and medial-lateral directions and polar moment of inertia) were calculated. Voxels identified as bone marrow and muscle were applied to fat (C) and water (D) images to determine bone marrow and intramuscular fat concentration [25].

Voxels representing muscle and bone marrow in the T1-weighted images were then used to identify the bone marrow and muscle voxels in the corresponding fat and water images. The fat concentration within the bone marrow of the midtibia and the muscles (i.e., intramuscular fat concentration) at the same level was determined by using the signal intensity (SI) from the fat image, the SI from the water image and the following equation: fat concentration = [fat SI / (fat SI + water SI)] \* 100 [25]. Fat content estimated using MRI and the IDEAL sequence is highly correlated with fat content measured using magnetic resonance spectroscopy in phantoms ( $r^2 = 0.985$ ) [26] and human vertebrae ( $r^2 = 0.904$ ) [27].

Intermuscular, subfascial and subcutaneous AT volume were estimated using the same custom software and the T1-weighted images used to assess bone architecture, bone strength, muscle volume and AT volume. Using a procedure similar to the procedure used for the mid thigh [8], subcutaneous AT was separated from intermuscular and subfascial AT by manually tracing a line over the crural fascia. Intermuscular AT was separated from subfascial AT and bone marrow by manually tracing a line on the outside border of the cortical bone and another line on the inside border of skeletal muscle. In areas where muscles separated, the line was drawn midway between the muscle borders. Thus, subcutaneous AT was the volume of AT between the skin and the crural fascia. Subfascial AT was considered the volume of AT between the deep crural fascia and the skeletal muscle border. Intermuscular AT was considered the volume of AT between the subfascial AT and the bone marrow.

The test-retest reliability for AT measures in the lower extremity using MRI was assessed previously in a combined sample of children with CP and typically developing children tested twice on separate days or on the same day after repositioning [8].

Intraclass correlation coefficients  $> 0.99$  and coefficients of variation between 0.6 and 3.4 % indicated excellent reliability. The test-retest reliability of midtibia bone marrow fat and midleg intramuscular fat concentrations from MRI were assessed in four typically developing children and four children with CP (5 to 11 years of age) tested on two separate days, six months apart. The intraclass correlations were 0.96 and  $> 0.99$  and the coefficients of variation were 0.04 % and 0.3 % for bone marrow and intramuscular fat concentrations, respectively.

### **3.3.6** *Statistical Analysis*

Using an effect size of 1.2 and setting the alpha at 0.05 and power at 0.8, a minimum sample size of 10 participants per group was estimated to determine if there were group differences in measures of bone architecture and bone marrow and muscle fat infiltration. Data were analyzed by using SPSS version 24.0 (IBM Corp, Armonk, NY). Descriptive statistics were conducted for all variables to screen for outliers and to assess normality. Independent sample t-tests were used to determine if there were group differences. The Mann-Whitney U test was used to assess differences in Tanner stage. To adjust all outcome measures of bone, muscle, AT and fat concentration for tibia length, a one-way analysis of covariance was used with tibia length as a covariate. Due to the equal number of boys and girls in each group, and the small number of girls included in the study ( $n = 4/\text{group}$ ), sex differences were not considered in the statistical analysis. Data are reported as means  $\pm$  SD in the text and tables. Data are reported as means  $\pm$  SE in the figures. The magnitude of the effects were determined using Cohen's  $d$  ( $d = \text{mean difference between groups/pooled SD}$ ), with 0.2, 0.5 and 0.8 representing small, moderate and large effect sizes, respectively [28].

### 3.4 Results

Twenty-three children with CP who met the inclusion criteria were invited to participate in the study. Eighteen children with CP enrolled in the study. Two children were unable to complete any MRI testing due to claustrophobia. One child was unable to remain still for the MRI testing in which the fat concentration of the bone marrow and muscle were determined due to attention deficit hyperactivity disorder and an inability to follow directions. Twelve typically developing children who met the inclusion criteria and matched a child with CP for age ( $\pm 1.2$  y), sex ( $n = 4$  girls and 8 boys per group) and race ( $n = 1$  white Hispanic, 10 white non-Hispanic and 1 Asian per group) were enrolled in the study as controls. All controls completed all testing. Data for the 12 matched pairs are presented. Two of the children with CP had a previous fracture (finger,  $n = 1$  and foot/ankle,  $n = 1$ ) and 3 were taking antiepileptic medication. None of the controls reported a previous fracture or chronic medication use. All data were normally distributed except subfascial and intermuscular AT volume, which were log transformed before statistical analyses were conducted.

Physical characteristics of the participants are shown in **Table 1**. Of the 12 children with CP, 8 children were considered GMFCS I and 4 children were considered GMFCS II. There were no group differences in age, pubic hair Tanner stage, testicular-penile/breast Tanner stage, height, body mass, body mass percentile, BMI, BMI percentile or tibia length (all  $p > 0.05$ ). However, children with CP compared to controls had lower height percentile ( $p < 0.05$ ). When compared to the 50<sup>th</sup> age- and sex-based percentiles, height percentile was lower in children with CP ( $p = 0.003$ ), but not different in controls ( $p = 0.531$ ). When compared to the 50<sup>th</sup> age- and sex-based percentiles for body mass and BMI, there were no differences in children with CP or controls (all  $p > 0.20$ ). Group comparisons of physical activity are also presented in **Table 1**. Children

with CP had 44 % fewer physical activity counts at the ankle compared to controls ( $p < 0.05$ ).

Table 3.1. Characteristics of children with cerebral palsy (CP) and typically developing children (Con).

|                              | CP<br>(n = 12)    | Con<br>(n = 12)   | <i>p</i>     | <i>d</i> |
|------------------------------|-------------------|-------------------|--------------|----------|
| Age (y)                      | 8.8 ± 2.1         | 8.8 ± 2.0         | 0.971        | 0.015    |
| Tanner Stage (I/II/III)      |                   |                   |              |          |
| Pubic hair                   | 8/2/2             | 11/1/0            | 0.291        | 0.767    |
| Testicular-Penile/Breast     | 7/5/0             | 10/1/1            | 0.378        | 0.293    |
| Height (m)                   | 1.24 ± 0.11       | 1.32 ± 0.11       | 0.106        | 0.689    |
| Height (%)                   | 19 ± 23           | 50 ± 28           | <b>0.006</b> | 1.232    |
| Body mass (kg)               | 26.6 ± 8.2        | 30.0 ± 7.4        | 0.293        | 0.441    |
| Body mass (%)                | 34 ± 33           | 55 ± 25           | 0.094        | 0.720    |
| BMI (kg/m <sup>2</sup> )     | 17.0 ± 3.4        | 17.0 ± 2.6        | 0.966        | 0.018    |
| BMI (%)                      | 50 ± 36           | 53 ± 28           | 0.872        | 0.067    |
| Tibia length (m)             | 0.273 ± 0.036     | 0.293 ± 0.031     | 0.173        | 0.577    |
| GMFCS (I/II)                 | 8/4               | -                 |              |          |
| Physical activity (counts/d) | 348,366 ± 197,001 | 628,044 ± 147,499 | <b>0.001</b> | 1.624    |

Values are means ± SD. % reflects the percentile relative to age- and sex-based norms; BMI = body mass index; GMFCS = Gross Motor Function Classification System. Significant differences are bolded.

Group comparisons of bone architecture at the level of the midtibia are shown in **Table 2**. Children with CP had 30 % lower cortical volume ( $p < 0.05$ ) than controls with no differences observed in medullary volume ( $p > 0.05$ ). Although marginally insignificant ( $p = 0.063$ ), children with CP had 25 % lower total bone volume than controls. Cortical width was 16 % thinner in the posterior portion and 32 % thinner in the medial portion of the shaft in children with CP compared to controls ( $p < 0.05$ ). There were no group differences observed in the anterior or lateral portions of the bone ( $p > 0.05$ ). Although marginally insignificant ( $p = 0.058$ ), children with CP displayed

17 % lower medullary width in the medial-lateral direction compared to controls. No group difference in medullary width was observed in the anterior-posterior direction ( $p > 0.05$ ). Children with CP displayed 15 % lower total bone width in the medial-lateral direction compared to controls ( $p < 0.05$ ), but no difference was observed in the anterior-posterior direction ( $p > 0.05$ ). When tibia length was used as a covariate, the differences remained statistically significant for all bone architecture measures ( $p < 0.05$ ), except for cortical width in the posterior portion of the bone ( $p > 0.05$ ).

Table 3.2. Bone architecture in the midtibia of children with cerebral palsy (CP) and typically developing children (Con).

|                                      | CP<br>(n = 12) | Con<br>(n = 12) | <i>p</i>     | <i>d</i> | <i>p</i> *   |
|--------------------------------------|----------------|-----------------|--------------|----------|--------------|
| Cortical volume (cm <sup>3</sup> )   | 12.3 ± 4.7     | 17.6 ± 5.8      | <b>0.022</b> | 1.009    | <b>0.012</b> |
| Medullary volume (cm <sup>3</sup> )  | 7.7 ± 3.7      | 9.0 ± 3.0       | 0.343        | 0.398    | 0.705        |
| Total bone volume (cm <sup>3</sup> ) | 20.0 ± 8.2     | 26.6 ± 8.4      | 0.063        | 0.800    | 0.119        |
| Cortical width (cm)                  |                |                 |              |          |              |
| Anterior                             | 0.41 ± 0.10    | 0.43 ± 0.11     | 0.656        | 0.184    | 0.434        |
| Posterior                            | 0.32 ± 0.07    | 0.38 ± 0.06     | <b>0.023</b> | 1.004    | 0.070        |
| Medial                               | 0.26 ± 0.04    | 0.38 ± 0.10     | <b>0.002</b> | 1.770    | <b>0.002</b> |
| Lateral                              | 0.27 ± 0.06    | 0.25 ± 0.07     | 0.586        | 0.227    | 0.397        |
| Medullary width (cm)                 |                |                 |              |          |              |
| Anterior-posterior                   | 0.51 ± 0.10    | 0.51 ± 0.08     | 0.981        | 0.010    | 0.061        |
| Medial-lateral                       | 0.52 ± 0.07    | 0.59 ± 0.09     | 0.058        | 0.820    | 0.199        |
| Total bone width (cm)                |                |                 |              |          |              |
| Anterior-posterior                   | 1.24 ± 0.22    | 1.32 ± 0.23     | 0.376        | 0.369    | 0.525        |
| Medial-lateral                       | 1.04 ± 0.11    | 1.22 ± 0.16     | <b>0.006</b> | 1.262    | <b>0.015</b> |

Values are means ± SD. *d* = the effect size of the group differences before tibia length was included as a covariate. \*Group difference *p* value when statistically controlled for tibia length using analysis of covariance. Significant differences are bolded.

Group comparisons of bone strength estimates at the level of the midtibia are shown in **Figure 2**. Children with CP had 34 % lower Zml ( $d = 1.127$ ,  $p = 0.012$ ) and

39 % lower J ( $d = 1.001$ ,  $p = 0.023$ ) compared to controls. Although marginally insignificant, children with CP had 29 % lower Zap ( $d = 0.822$ ,  $p = 0.057$ ) compared to controls. When tibia length was used as a covariate, the significant differences in Zml and J remained statistically significant ( $p = 0.017$  and  $0.035$ , respectively). **Figure 3** shows a higher bone marrow fat concentration in children with CP compared to controls ( $82.1 \pm 1.8$  % vs.  $80.5 \pm 1.9$  %;  $d = 0.909$ ,  $p = 0.037$ ), which remained significantly higher when tibia length was used as a covariate ( $p = 0.004$ ).

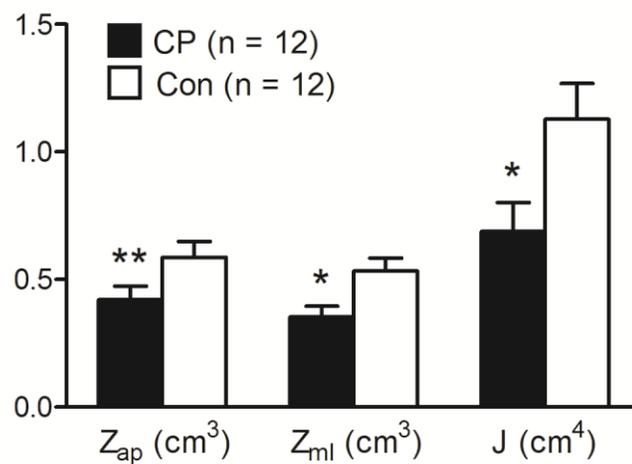


Figure 3.2. Estimates of bone strength [section modulus in the anterior-posterior (Zap) and medial-lateral (Zml) directions and polar moment of inertia (J)] in the midtibia of children with cerebral palsy (CP) and typically developing children (Con). Values are means  $\pm$  SE. \*Group difference,  $p < 0.05$ . \*\*Group difference,  $p = 0.057$ .

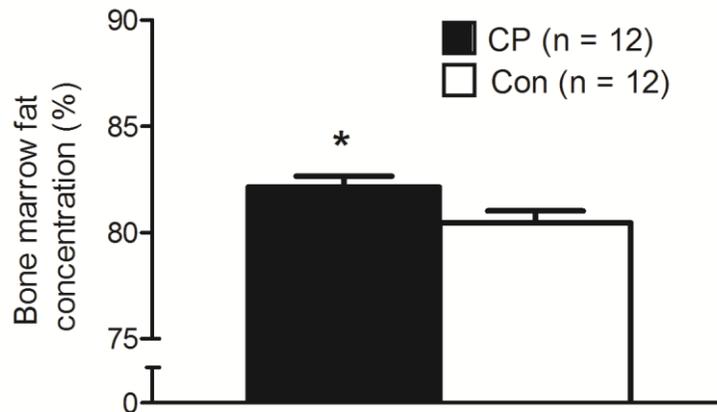


Figure 3.3. Bone marrow fat concentration (%) in the midtibia of children with cerebral palsy (CP) and typically developing children (Con). Values are mean  $\pm$  SE. \*Group difference,  $p < 0.05$ .

Group comparisons of muscle volume and AT volume in the midleg are shown in **Table 3**. Children with CP had 39 % lower muscle volume and 3.3 fold higher subfascial AT volume compared to controls (both  $p < 0.05$ ). There were no group differences in subcutaneous or total AT volume between groups ( $p > 0.05$ ). Although marginally insignificant ( $p = 0.077$ ), children with CP had 3.7 fold lower intermuscular AT than controls. The differences in muscle volume and subfascial AT volume remained statistically significant when tibia length was used as a covariate ( $p < 0.05$ ). Moreover, a significantly higher intermuscular AT in children with CP than controls emerged when tibia length was used as a covariate ( $p < 0.05$ ). **Figure 4** shows a higher intramuscular fat concentration in children with CP compared to controls ( $25.0 \pm 8.0$  % vs.  $16.1 \pm 3.3$  %;  $d = 1.587$ ,  $p = 0.003$ ), which remained significantly higher when tibia length was used as a covariate ( $p = 0.002$ ).

Table 3.3. Muscle and adipose tissue (AT) volume in the midleg of children with cerebral palsy (CP) and typically developing children (Con).

|  | CP<br>(n = 12) | Con<br>(n = 12) | <i>p</i>     | <i>d</i> | <i>p</i> *   |
|--|----------------|-----------------|--------------|----------|--------------|
| Muscle volume (cm <sup>3</sup> )             | 144.8 ± 56.4   | 237.1 ± 70.0    | <b>0.002</b> | 1.461    | <b>0.001</b> |
| Intermuscular AT volume (cm <sup>3</sup> )** | 5.1 ± 7.0      | 1.4 ± 1.1       | 0.077        | 0.787    | <b>0.036</b> |
| Subfascial AT volume (cm <sup>3</sup> )**    | 4.2 ± 5.4      | 1.3 ± 0.7       | <b>0.008</b> | 1.229    | <b>0.002</b> |
| Subcutaneous AT volume (cm <sup>3</sup> )    | 119.5 ± 58.3   | 140.6 ± 43.3    | 0.325        | 0.415    | 0.868        |
| Total AT volume (cm <sup>3</sup> )           | 128.8 ± 66.2   | 143.3 ± 44.7    | 0.537        | 0.261    | 0.796        |

Values are means ± SD. *d* = the effect size of the group differences before tibia length was included as a covariate. \*Group difference *p* value when statistically controlled for tibia length using analysis of covariance. \*\*Group difference *p* value and *d* for the log transformed data are reported, but means ± SD for untransformed data are presented for easier comparisons among the AT depots. Significant differences are bolded.

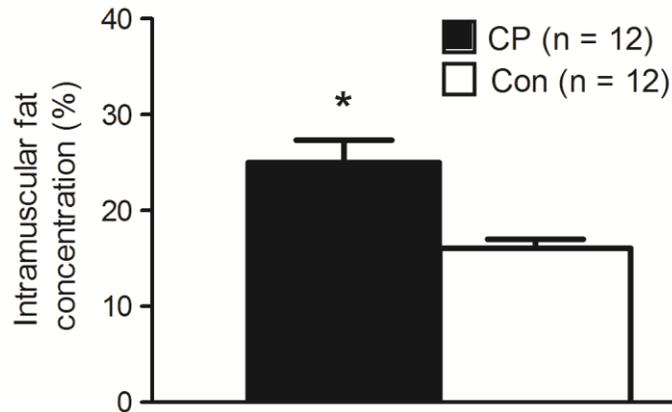


Figure 3.4. Intramuscular fat concentration (%) in the midtibia of children with cerebral palsy (CP) and typically developing children (Con). Values are mean ± SE. \*Group difference, *p* < 0.05.

A visual depiction of the bone, muscle and fat contrast between a boy with CP and a typically developing control boy of similar height and the same tibia length is shown in **Figure 5**.

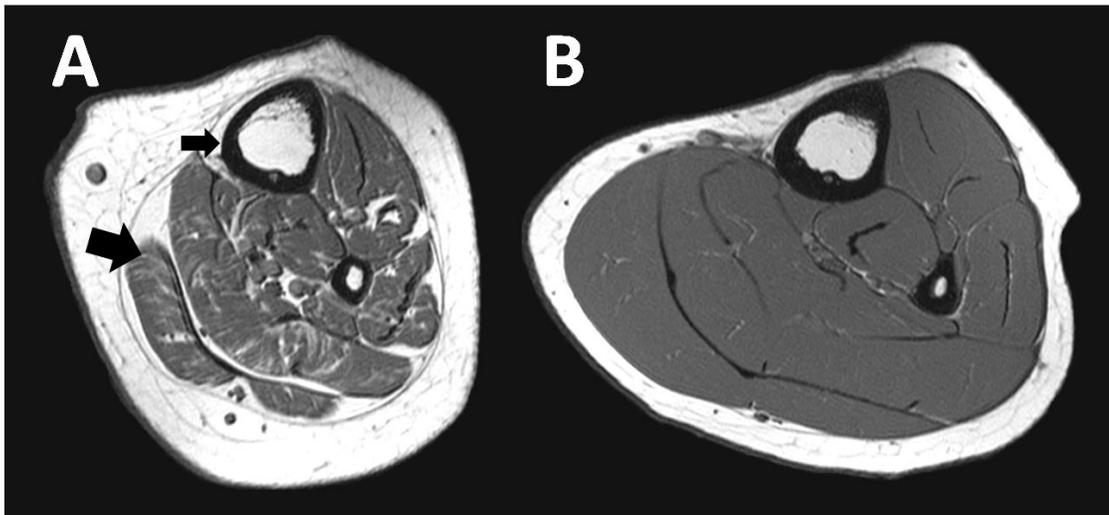


Figure 3.5. Raw T1-weighted magnetic resonance images from the midtibia demonstrate the marked deficit in bone architecture and muscle volume and the high infiltration of fat within and around the musculature in an ambulatory boy with mild CP (A) compared to a typically developing boy with the same tibia length (B). In the image of the child with CP (A), the small black arrow highlights the thin cortical shell and the large arrow highlights the fat infiltration of muscle.

### 3.5 Discussion

This is the first study to report that ambulatory children with mild spastic CP relative to typically developing children have markedly underdeveloped bone architecture and lower estimates of bone strength. It is also the first study to report that ambulatory children with mild spastic CP have elevated fat infiltration of bone marrow and skeletal muscle. The finding that bone marrow is infiltrated with fat in children with mild CP is particularly novel because, to our knowledge, it has not been examined in individuals with CP, irrespective of level of involvement or age. Together the

findings suggest that the underdevelopment of bone architecture and fat infiltration of the musculoskeletal system in nonambulatory children with more severe forms of CP is also present in ambulatory children with milder forms of the disorder. The findings are concerning because poor bone architecture and an elevated bone marrow adiposity are associated with an increased risk of fracture [13-15]. Moreover, elevated bone marrow and muscle adiposity are linked to impaired glucose tolerance [12, 16, 17] and cardiometabolic disease [18].

The finding that bone architecture is underdeveloped and bone strength is lower in ambulatory children with mild CP is consistent with previous studies in children [4, 6] and adults [29, 30] with reduced mechanical loading. However, the magnitude of the deficit is surprising. In the current study, cortical volume,  $Z$  in the medial-lateral plane and  $J$  were  $\geq 30$  % lower in the midtibia of ambulatory children with mild CP vs. controls. These estimated deficits are approximately half those observed in the midfemur of nonambulatory children with CP [4], but generally worse than the 15-31 % estimated bone deficits reported in the tibial [30] and femoral shaft [29, 30] of adults with complete spinal cord injury (SCI). The smaller deficits in the ambulatory children with CP observed in the present study compared to the deficits observed in a previous study of nonambulatory children with CP [4] is not surprising because ambulatory children with CP experience mechanical loading while they are walking or running. Moreover, although physical activity was 44% lower in ambulatory children with CP compared to typically developing children matched for age, sex and race in the present study, it was not as dramatic as the discrepancy (70 % lower) previously reported for nonambulatory children with CP [4]. The generally larger bone strength deficits observed in ambulatory children with CP than the deficits previously reported for adults

with SCI is likely due to the onset of the condition. In children with CP, the restriction of mechanical loading begins at birth or shortly afterward and continues throughout life. Therefore, the mechanical loading needed for optimal cortical expansion at the subperiosteal surface is reduced or absent and the total width of the bone does not reach its capacity. On the other hand, because SCI usually occurs during adulthood, the restriction of mechanical loading usually begins after growth and cortical expansion is complete or nearly complete. Therefore, the total width of the bone reaches its capacity [29, 30]. The deficit in bone strength in adults with SCI results from increased bone remodeling and endocortical resorption, which has a smaller effect on bone strength than restricted subperiosteal expansion [31]. The difference in timing of physical activity restriction highlights the importance of mechanical loading during childhood to optimize cortical expansion and the strength of bone. Together, findings from the current study and previous studies suggest that ambulatory children with mild CP have considerable bone strength deficits that need clinical attention. A handful of studies suggest that treatments, such as high-frequency, low-magnitude vibration [33, 34], may aid in addressing such bone deficits.

The observation that ambulatory children with mild CP have elevated fat infiltration of muscle in the midleg, as indicated by elevated subfascial AT and intramuscular fat concentration, is consistent with the observation in nonambulatory children with moderate to severe CP [8] and young adults with mild to moderate CP [35]. Using magnetic resonance imaging, Johnson et al. [8] reported a 2.3-fold and 1.7-fold higher intermuscular and subfascial AT area in the mid thigh of nonambulatory children with CP compared to typically developing children. These AT depots were negatively correlated with physical activity ( $r = -0.76$  and  $r = -0.63$ , respectively).

Furthermore, Noble and colleagues [35] reported greater intramuscular fat in the leg muscles in young adults with CP compared to controls. In the present study, physical activity assessed using physical activity monitors was 44 % lower in children with mild CP than controls. There is also evidence that gait declines in 25 % of children with CP as they become adults [1]. The sedentary behavior and reduced mechanical loading observed with CP is also observed with SCI. Individuals with SCI, concomitant with the altered skeletal metabolism, experience vast muscular atrophy, increased muscular adiposity, insulin resistance and an increased prevalence of type 2 diabetes mellitus [12, 36]. On the other hand, the sedentary behavior and reduced mechanical loading in individuals with CP occurs at or near the time of birth, rather than it being acquired later in life. Therefore, the musculoskeletal metabolic dysregulation in children with CP, resulting in losses of normal muscle function, likely leads to an enhanced risk for cardiometabolic disease [18]. This idea is supported by the 2-to-3 fold greater mortality rate from coronary heart disease in individuals with CP compared to the general population [37]. Therefore, ameliorating regressive behavior in physical activity, which is apparent even in children with a mild form of the disorder, is imperative to the health of the musculature, and minimizing the risk of cardiometabolic disease in individuals with CP.

The novel finding of elevated fat concentration in the bone marrow of children with CP in the present study could be viewed as somewhat surprising because the children were ambulatory with a mild form of CP, the children averaged only 8.8 years of age and their BMI was not different from the 50th age-based percentile; however, the finding is consistent with their underdeveloped bone architecture and low estimates of bone strength relative to controls. Moreover, the finding is consistent with the elevated

fat concentration in the bone marrow of other groups with limited mobility [11, 14, 38]. Bone marrow-derived mesenchymal progenitor cells have a multipotency to differentiate into an osteogenic or adipogenic lineage while concomitantly suppressing the opposed lineage [39]. Recently, it has been reported that bone marrow fat concentration is inversely related to cortical bone area in adolescents 15 to 20 years of age [40] and adults [40, 41]. There is evidence suggesting that physical activity governs the extent of bone marrow AT. Rantalainen et al. [42] reported that female athletes had significantly greater cortical area, strength strain index and bone marrow density (indicative of lower marrow adiposity) in the midtibia compared to healthy controls. On the other hand, it has been suggested that adults with SCI have a higher amount of yellow vs. red bone marrow AT compared to healthy controls [11]. The higher proportion of yellow marrow may be explained by the metabolic shift in mesenchymal progenitor cell differentiation away from osteogenesis and towards adipogenesis at the endocortical surface due to the reduced mechanical loading observed after an SCI. In the current study, children with mild CP had a deficit in cortical volume and bone strength, but they had no significant difference in the volume of the medullary cavity. Therefore, the connection between the higher concentration of bone marrow adiposity and the deficit in bone macroarchitecture in ambulatory children with mild CP relative to typically developing children and the clinical implications of the relationship requires further investigation.

The present study has several strengths. First, there was very careful attention paid to the selection of the research participants which limited the within group variability and the need for a large sample size. Specifically, all children with CP had spasticity, were ambulatory and had a mild form of the disorder. The typically

developing children were not different from the children with CP in age, sex, race or sexual maturity. Furthermore, the typically developing children were not different from the 50<sup>th</sup> age- and sex-based percentiles for height, body mass and BMI. A second strength of the present study was that most of the deficits in bone architecture and bone strength and all of the elevated estimates of fat infiltration of bone marrow and muscle in the children with CP remained when small, insignificant differences in tibia length were statistically controlled. A third strength of the study was the use of MRI to assess different AT and fat depositions. Magnetic resonance imaging is considered the gold standard in vivo imaging modality for assessing soft tissue [44], it provides valid estimates of bone architecture [45] and fat concentration [26] and it does not expose participants to ionizing radiation, as does computed tomography. Furthermore, the reliability of the measures in the present study was excellent, with intraclass correlations for repeat testing  $\geq 0.96$  for all MRI measures of bone architecture, bone marrow and muscle fat concentration and muscle volume. Lastly, physical activity was estimated using accelerometer-based activity monitors that have been validated with a wide range of both gross and fine motor physical activities [43] and have good reliability for assessment in children with CP, as reported in the present study. The assessment of physical activity allowed for a broader mechanistic approach to understanding the underdeveloped musculoskeletal system and increased fat infiltration within muscle and bone in a group of ambulatory children with mild spastic CP.

The limitations of the study must also be discussed. One limitation is the small sample size. Therefore, the results should be interpreted with caution. Studies of children with CP using MRI are challenging because the children often have difficulty holding still due to spasticity and/or behavioral issues, and they often have noise

sensitivity and cognitive issues. Moreover, the cost of MRI studies limits the number of participants that can be enrolled. However, even with these challenges, significant differences in cortical volume, bone widths, estimates of bone strength, bone marrow fat concentration, muscle volume, subfascial AT volume and intramuscular fat concentration were observed. This is attributed to the magnitude of the differences as well as the robustness of the measures. As previously noted, the reliability of the MRI assessment of bone architecture, muscle volume and fat infiltration measures used in the present study is extremely good. Furthermore, a set of identical twins was included in the study and their data reflect the group differences. For example, cortical volume, muscle volume, and bone marrow and intramuscular fat concentration were 30 % lower, 39 % lower, 2 percentage points higher, and 9 percentage points higher, respectively, in the children with CP compared to the typically developing children. The same measurements were 18 % lower, 39 % lower, 2 percentage points higher and 7 percentage points higher, respectively, in the twin with CP (5.5 years of age, GMFCS I) compared to the typically developing twin. A second study limitation is the absence of glucose tolerance, insulin sensitivity and inflammatory markers. It has been established that elevated AT surrounding muscle and fat within muscle are associated with a disturbance of euglycemic conditions [12] and insulin resistance [16], as well as promoting a pro-inflammatory environment [46]. Whether this relationship exists in children with mild CP and if elevated bone marrow fat is associated with a disturbance in these metabolic markers warrants further investigation.

Taken together, the findings from the present study suggest that ambulatory children with mild spastic CP compared to typically developing children have underdeveloped bone architecture, low bone strength and a greater fat infiltration of the

bone marrow in the midtibia and the surrounding leg musculature. The underdeveloped bone architecture and fat infiltration may be related to the low level of physical activity potentially promoting an intramuscular pro-inflammatory environment and blunting osteogenesis while favoring adipogenesis within the bone marrow cavity. Additional studies that determine whether the adverse musculoskeletal fat profile is associated with a disturbance in glucose homeostasis or insulin function are needed. Studies that examine whether increased physical activity or other interventions would enhance the bone architecture while reducing the observed infiltration of fat in the musculoskeletal system of children with mild CP are also needed.

### **3.6 Conflicts of Interest**

Daniel G. Whitney, Harshvardhan Singh, Freeman Miller, Mary F. Barbe, Jill M. Slade, Ryan T. Pohlig and Christopher M. Modlesky declare that they have no conflicts of interest.

### **3.7 Acknowledgements**

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## Chapter 4

### **LEG MUSCLE PROFILE OF INDIVIDUAL VOLUME DEFICITS AND HIGHER FAT CONCENTRATION IN AMBULATORY CHILDREN WITH SPASTIC CEREBRAL PALSY AND THE RELATIONSHIP WITH MUSCLE STRENGTH**

#### **4.1 Abstract**

**AIM:** Children with cerebral palsy (CP) have a small and weak musculature. The purpose of this study was to determine if leg intramuscular fat concentration is negatively related to ankle muscle strength in ambulatory children with spastic CP.

**METHODS:** Fourteen ambulatory children with spastic CP ( $8.1 \pm 2.5$  yrs;  $n = 10$  boys) and 14 sex- and age-matched controls participated. Magnetic resonance imaging determined muscle volume and fat concentration of all leg muscles. Plantar flexor (PF) and dorsi flexor (DF) strength was assessed using dynamometry.

**RESULTS:** Compared to controls, children with CP had lower PF and DF strength ( $p < 0.01$ ), volumes of most muscles, even after statistically controlling for tibia length ( $p \leq 0.05$ ), blunted relationships between tibia length and volume of the flexor digitorum longus and tibialis anterior (interaction, both  $p < 0.05$ ) and elevated fat concentration of all muscles ( $p < 0.02$ ). For a given muscle volume of the gastrocnemius and soleus and for the tibialis anterior, children with CP had weaker PF and DF strength, respectively, compared to controls ( $p < 0.05$ ). When muscle volume was corrected for intramuscular fat, the relationships between PF with gastrocnemius and soleus and between DF with tibialis anterior became stronger.

INTERPRETATION: Compared to typically developing children, children with CP have small leg muscles accompanied by elevated intramuscular fat. The growth of the flexor digitorum longus and tibialis anterior may be restricted to a greater degree than other leg muscles in children with CP. The elevated intramuscular fat contributed to the weaker muscle strength, but only to a small degree.

## **4.2 Introduction**

Cerebral palsy (CP) results from damage to the brain around the time of birth and is associated with small and weak skeletal muscles [1, 2]. Poor muscle function contributes to the low levels of physical activity [3] and poor cardiorespiratory fitness observed in children with CP [4]. Importantly, as these children age into their adult years, the majority will experience a further deterioration of mobility [5]. Therefore, interventions aimed towards improving muscle strength and function are needed for this population.

Muscle size is an important component of muscle strength and function in children with CP [1]. Specific muscle tension (force per unit of muscle) of the leg muscles are lower in children with CP with the degree of specific muscle tension deficit varying based on anatomical location. This suggests a hindered ability to activate muscle tissue which may be more impacted in certain muscles or regions of muscle of the leg. The discordance between muscle size and strength is likely related to impaired neurological features and differences in mechanical and transcriptional elements at the level of the muscle. Although, elevated intramuscular fat is also associated with weaker muscle fibers in adults [6]. We have previously reported that ambulatory [7] and nonambulatory [3] children with CP have elevated skeletal muscle fat infiltration of the

lower extremity. To date, the contribution of intramuscular fat on ankle muscle strength has not been assessed in children with CP. Moreover, in vivo imaging procedures used to quantify muscle size in children may not be accounting for the degree of intramuscular fat accumulation. Therefore, the discordant relationship between muscle size and strength may not be as pronounced once the elevated intramuscular fat is accounted for.

Detailing individual muscle volumes and intramuscular fat content of the leg in children with CP is necessary to better understand the unique underdeveloping musculature and potential mechanisms of muscle dysfunction. This will lead to better target-specific interventions focused on the more affected muscle(s) for improving muscle function, mobility and favorable health outcomes in children with CP. The primary objective of this study was to determine the pattern of muscle volume and intramuscular fat infiltration of individual leg muscles in ambulatory children with spastic CP. The secondary objective of this study was to determine the muscle size-strength relationship of the ankle and if intramuscular fat infiltration is negatively related to muscle strength of the ankle in ambulatory children with spastic CP. We hypothesized that ambulatory children with spastic CP will have weaker muscle strength of the ankle and will have lower muscle volume and elevated intramuscular fat concentration of all individual leg muscles compared to controls. We further hypothesized that the discordant relationship between muscle size and strength will be partially explained by elevated intramuscular fat concentration of the gastrocnemius and soleus with plantar flexor force and the tibialis anterior with dorsi flexor force.

### **4.3 Materials and Methods**

#### **4.3.1 *Participants***

Children with spastic CP (n = 10 boys and 4 girls), between the ages of 4 and 11 years and that could ambulate independently (classified as I or II from the Gross Motor Function Classification System [GMFCS] by a physician assistant) were recruited from the AI duPont Hospital for Children in Wilmington, DE. Typically developing children were matched to children with CP for sex, age and race, that fell between the 5<sup>th</sup> and 95<sup>th</sup> percentile for height and body mass and had no previous history of chronic medication use. The Institutional Review Boards at the AI duPont Hospital for Children and the University of Delaware approved the study procedures. Prior to testing, written consent and assent was obtained by the legal guardians and the participants, respectively.

#### **4.3.2 *Muscle Strength***

Plantar flexor (PF) and dorsi flexor (DF) strength was measured by a Biodex System-3 dynamometer (Biodex Medical Systems, Inc., Shirley, NY) in the more affected leg in children with CP and in the nondominant leg in controls. The force plate was set to 15<sup>0</sup> plantar flexion and the knee angle was less than 20<sup>0</sup> of flexion. The participants performed three sets of unilateral maximal voluntary isometric contractions separated by 30 seconds of rest. The highest value is reported.

#### **4.3.3 *Magnetic Resonance Imaging***

Magnetic resonance imaging (General Electric, 1.5 T, Milwaukee, WI) was used to assess muscle volume and fat concentration of individual muscles along the length of the tibia in the more affected leg in children with CP and in the non-dominant leg in

controls. All participants were immobilized from the waist down using the BodyFIX system (Medical Intelligence, Inc., Schwabmünchen, GER), as previously described [8]. Axial images were collected from the tibia plateau to the inferior malleolar articular surface (0.5 cm thick separated by 0.5 cm of spacing) using a semiflex long bone array coil (ScanMed, Omaha, NE) and two different sequences yielding 1) T1-weighted images and 2) fat and water images, as previously described [9]. Briefly, T1-weighted images were filtered using a median filter and voxels were segmented and assigned to muscle and adipose tissue.

Images along the length of the tibia were processed by a single research assistant blind to participant group using software developed with Interactive Data Language (Research Systems, Inc, Boulder, CO). Individual muscles were manually traced over the muscle boundary on raw T1-weighted images (**Figure 1A and B**). The volume in the full image set was summed for each muscle which excluded voxels assigned to adipose tissue. Individual muscle regions identified from the T1-weighted images were used to identify the individual muscles in the corresponding fat and water images (**Figure 1C and D**) to determine fat concentration as previously described [9]. The average fat concentration for each muscle along the length of the tibia is reported. The individual leg muscles in this study include the extensor digitorum longus, extensor hallicus longus, flexor digitorum longus, flexor hallicus longus, peroneus brevis, peroneus tertius, peroneus longus, tibialis posterior, tibialis anterior, gastrocnemius and soleus. Due to the difficulty of distinguishing between the extensor digitorum longus and extensor hallicus longus and between the peroneus brevis and peroneus tertius, each of these muscle pairs were combined and are reported as one muscle (i.e., extensor digitorum/hallicus longus and peroneus brevis/tertius). The inter- and intra-rater

reliability of all individual muscle volume and fat concentration along the length of the tibia using MRI was assessed in four children with CP and four typically developing children (5 to 11 years of age). Intraclass correlation coefficient values were  $> 0.96$  for muscle volume and fat concentration and the coefficients of variation ranged from 1.23 - 5.44 % for muscle volume and 1.41 - 4.49 % for fat concentration, indicating excellent reliability.

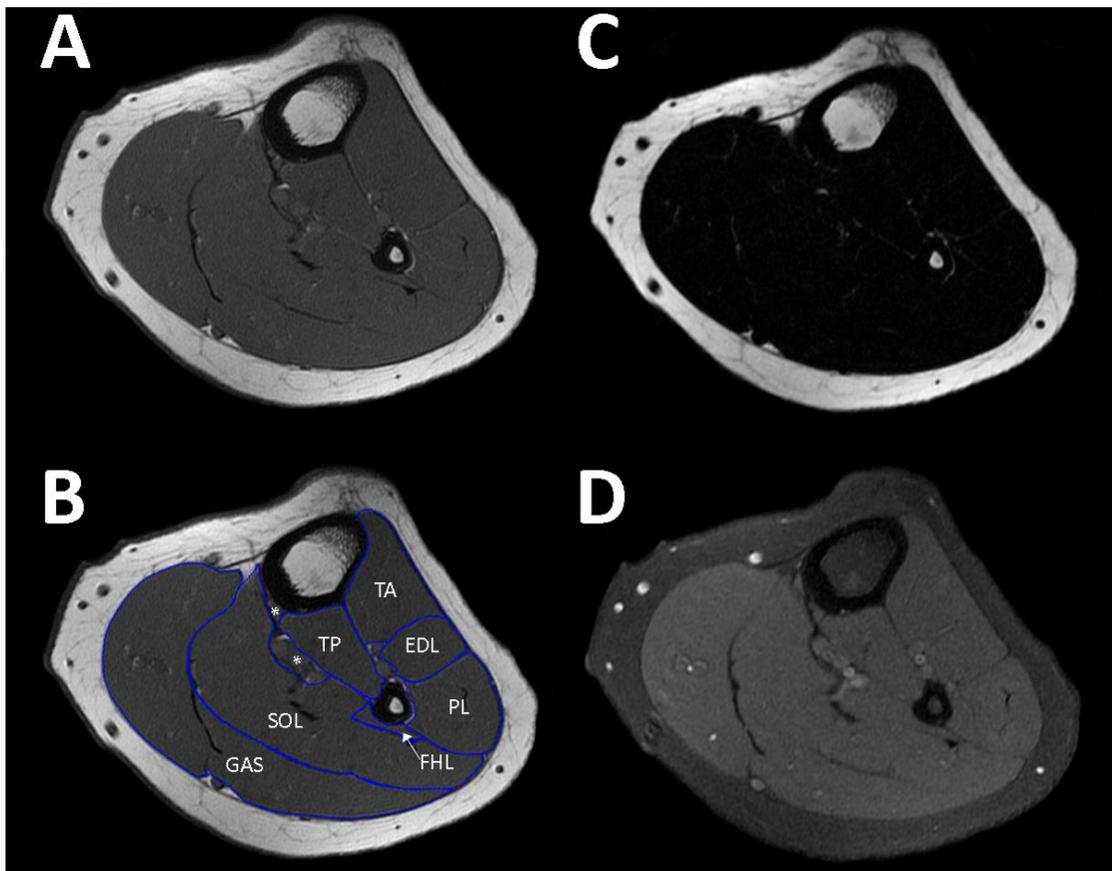


Figure 4.1. Raw T1-weighted magnetic resonance images (A) were used to identify individual muscles along the length of the tibia (B). This representative image, at ~30 % site distal to the proximal end of the tibia, shows seven of the eleven collected muscles. The four muscles not shown here originate more distally along the tibia. The blue lines separate the gastrocnemius (GAS), soleus (SOL), tibialis posterior (TP), tibialis anterior (TA), extensor digitorum longus (EDL), peroneus longus (PL) and flexor hallucis longus (FHL) muscles from one another and from blood vessels (\*). These regions were used to identify individual muscles on the corresponding fat (C) and water (D) image to determine intramuscular fat concentration.

#### 4.3.4 Statistical Analysis

Data were analyzed by SPSS version 24.0 (IBM Corp, Armonk, NY). All variables were checked for normality using skewness, kurtosis and the Shapiro-Wilk test. Group differences were determined using independent t tests if the data were normally distributed and Mann-Whitney U test if the data were not normally distributed. Unadjusted values are presented as mean  $\pm$  SD. The magnitude of the effect was determined using Cohen's *d* (*d*), with 0.2, 0.5 and 0.8 representing small, moderate and large effect sizes, respectfully [10]. Measures of individual muscle volumes were further tested between groups using one-way ANCOVA adjusted for tibia length. The interaction between tibia length and individual muscle volumes were tested to confirm homogeneity of regression between groups, and if there were no differences, the interaction was removed from the model.

Univariate regression analyses were performed to determine the relationships between gastrocnemius and soleus volume with PF strength and tibialis anterior volume with DF strength. These muscles were chosen because they are the primary muscles

involved in PF and DF strength, respectively. To determine the contribution of fat concentration on muscle strength of the ankle, the same univariate regression analyses were performed after correcting muscle volume for fat concentration using equation 1 (**Eq. 1**). The interaction between measures of muscle strength of the ankle and uncorrected and corrected muscle volumes were tested.

**Eq. 1** Corrected muscle volume (cm<sup>3</sup>) = muscle volume (cm<sup>3</sup>) – [muscle volume (cm<sup>3</sup>) x fat concentration (%)]

The ratio of PF to uncorrected and corrected gastrocnemius (PF<sub>gas</sub> and PF<sub>gas-fat</sub>, respectively) or soleus (PF<sub>sol</sub> and PF<sub>sol-fat</sub>, respectively) volume and DF to uncorrected and corrected tibialis anterior (DF<sub>ta</sub> and DF<sub>ta-fat</sub>, respectively) volume were assessed to further determine the contribution of fat concentration on muscle strength of the ankle.

#### **4.4 Results**

Physical characteristics and muscle strength are presented in **Table 1**. Seven children with CP were classified as GMFCS 1 and 7 children were GMFCS 2. There were no group differences in age, Tanner staging, height, body mass, BMI, BMI percentile or tibia length (all  $p > 0.05$ ). Children with CP had lower height percentile and body mass percentile (both  $p < 0.05$ ) compared to controls. Children with CP had 73 % and 71% weaker PF and DF strength, respectively, compared to controls (both  $p < 0.001$ ).

Table 4.1. Characteristics of children with cerebral palsy (CP) and typically developing children (Con).

|                          | CP<br>(n = 14) | Con<br>(n = 14) | <i>p</i>         | <i>d</i> |
|--------------------------|----------------|-----------------|------------------|----------|
| Physical characteristics |                |                 |                  |          |
| Age (y)                  | 8.1 ± 2.5      | 8.3 ± 2.3       | 0.854            | 0.070    |
| Sex (male/female)        | 10/4           | 10/4            |                  |          |
| Race (W/B/H/O)           | 10/2/2/0       | 10/2/1/1        |                  |          |
| Tanner Stage (1/2/3)     |                |                 |                  |          |
| Pubic hair               | 10/3/1         | 13/1/0          | 0.303            |          |
| Testicular-Penile/Breast | 9/5/0          | 12/1/1          | 0.129            |          |
| Height (m)               | 1.21 ± 0.14    | 1.29 ± 0.11     | 0.094            | 0.661    |
| Height (%)               | 19.4 ± 21.0    | 56.0 ± 29.1     | <b>0.001</b>     | 1.460    |
| Body mass (kg)           | 25.4 ± 9.7     | 29.1 ± 8.1      | 0.286            | 0.413    |
| Body mass (%)            | 33.6 ± 31.6    | 59.1 ± 25.8     | <b>0.027</b>     | 0.888    |
| BMI (kg/m <sup>2</sup> ) | 16.8 ± 3.6     | 17.1 ± 3.0      | 0.784            | 0.105    |
| BMI (%)                  | 50.4 ± 36.1    | 55.0 ± 29.9     | 0.714            | 0.141    |
| Tibia length (cm)        | 26.4 ± 4.0     | 28.2 ± 3.1      | 0.186            | 0.515    |
| GMFCS (1/2)              | 7/7            | -               |                  |          |
| Muscle strength          |                |                 |                  |          |
| Plantarflexion (Nm)      | 9.7 ± 9.0      | 35.2 ± 12.1     | <b>&lt;0.001</b> | 2.407    |
| Dorsiflexion (Nm)        | 3.5 ± 3.0      | 12.1 ± 4.9      | <b>&lt;0.001</b> | 2.200    |

W, white; B, black; H, hispanic; O, other; GMFCS; gross motor function classification system. Values are means ± SD. *d* = the effect size of the group differences. % reflects the percentile relative to sex- and age-based norms. Significant differences are bolded.

Muscle volume and fat concentration of individual muscles are presented in **Table 2**. Compared to controls, children with CP had lower volume of all muscles (all  $p < 0.05$ ), except for the marginally insignificant peroneus longus ( $p = 0.052$ ), ranging from 27 % (peroneus longus) to 44 % (tibialis anterior). The slopes for tibia length regressed on flexor digitorum longus volume and tibialis anterior volume were

significantly different between groups (interaction,  $p = 0.040$  and  $0.005$ , respectively).

**Figure 2** shows the blunted relationships in children with CP compared to controls. After adjusting for tibia length, all muscle volumes remained significantly lower in children with CP compared to controls (all  $p \leq 0.05$ ). Compared to controls, children with CP had elevated fat concentration of all muscles (all  $p < 0.05$ ) ranging from 4.4 (tibialis posterior) to 10.1 (soleus) percentile units.

Table 4.2. Individual muscle volume and fat concentration in children with cerebral palsy (CP) and typically developing children (Con).

|                                    | CP<br>(n = 14) | Con<br>(n = 14) | <i>p</i>         | <i>d</i> | <i>p</i> <sup>1</sup>         |
|------------------------------------|----------------|-----------------|------------------|----------|-------------------------------|
| <b>Volume (cm<sup>3</sup>)</b>     |                |                 |                  |          |                               |
| Extensor digitorum/hallicus longus | 23.4 ± 9.58    | 34.4 ± 10.8     | <b>0.008</b>     | 1.082    | <b>0.009</b>                  |
| Flexor digitorum longus            | 8.77 ± 3.42    | 13.6 ± 4.61     | <b>0.004</b>     | 1.199    | <b>0.004</b> <sup>2</sup>     |
| Flexor hallicus longus             | 17.8 ± 8.68    | 29.9 ± 10.1     | <b>0.002</b>     | 1.282    | <b>0.002</b>                  |
| Peroneus brevis/tertius            | 11.9 ± 7.38    | 18.6 ± 5.75     | <b>0.012</b>     | 1.028    | <b>0.026</b>                  |
| Peroneus longus                    | 21.7 ± 9.80    | 29.6 ± 10.8     | 0.052            | 0.771    | 0.165                         |
| Tibialis posterior                 | 30.7 ± 13.3    | 42.5 ± 13.1     | <b>0.027</b>     | 0.887    | <b>0.050</b>                  |
| Tibialis anterior                  | 25.1 ± 9.92    | 45.1 ± 14.9     | <b>&lt;0.001</b> | 1.610    | <b>&lt;0.001</b> <sup>2</sup> |
| Gastrocnemius                      | 66.2 ± 33.4    | 108 ± 36.4      | <b>0.004</b>     | 1.195    | <b>0.006</b>                  |
| Soleus                             | 88.1 ± 42.5    | 151 ± 50.0      | <b>0.001</b>     | 1.367    | <b>0.001</b>                  |
| <b>Fat concentration (%)</b>       |                |                 |                  |          |                               |
| Extensor digitorum/hallicus longus | 18.5 ± 6.08    | 11.8 ± 2.62     | <b>0.001</b>     | 1.538    | -                             |
| Flexor digitorum longus            | 21.1 ± 9.71    | 14.3 ± 2.47     | <b>0.012</b>     | 1.112    | -                             |
| Flexor hallicus longus             | 21.6 ± 8.67    | 12.9 ± 2.96     | <b>0.001</b>     | 1.499    | -                             |
| Peroneus brevis/tertius            | 19.7 ± 7.07    | 12.9 ± 3.19     | <b>&lt;0.001</b> | 1.332    | -                             |
| Peroneus longus                    | 23.1 ± 8.29    | 14.6 ± 3.25     | <b>0.001</b>     | 1.468    | -                             |
| Tibialis posterior                 | 17.2 ± 6.14    | 12.8 ± 1.91     | <b>0.006</b>     | 1.088    | -                             |
| Tibialis anterior                  | 20.1 ± 5.93    | 14.3 ± 3.47     | <b>0.001</b>     | 1.234    | -                             |
| Gastrocnemius                      | 21.2 ± 8.16    | 15.1 ± 4.65     | <b>0.014</b>     | 0.959    | -                             |
| Soleus                             | 23.8 ± 9.74    | 13.7 ± 3.10     | <b>0.001</b>     | 1.578    | -                             |

Values are means ± SD. *d* = the effect size of the group differences before tibia length was included as a covariate. <sup>1</sup>Group difference *p* value when statistically

controlled for tibia length using analysis of covariance. <sup>2</sup>See **Figure 2**. Significant differences are bolded.

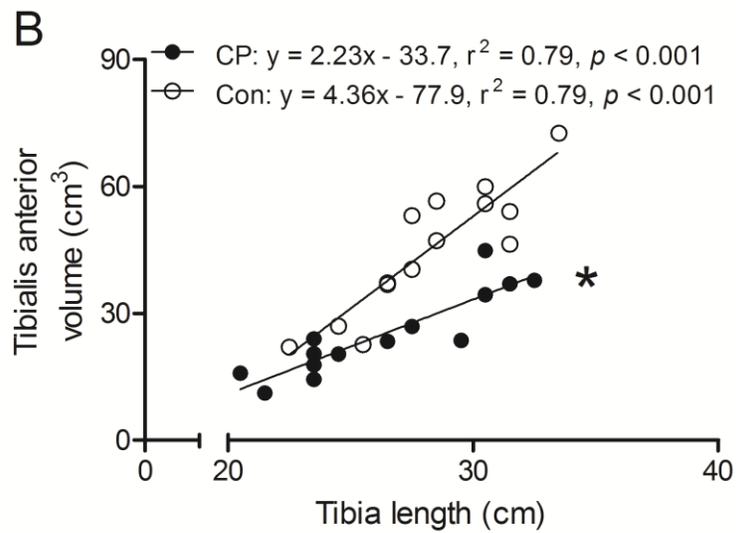
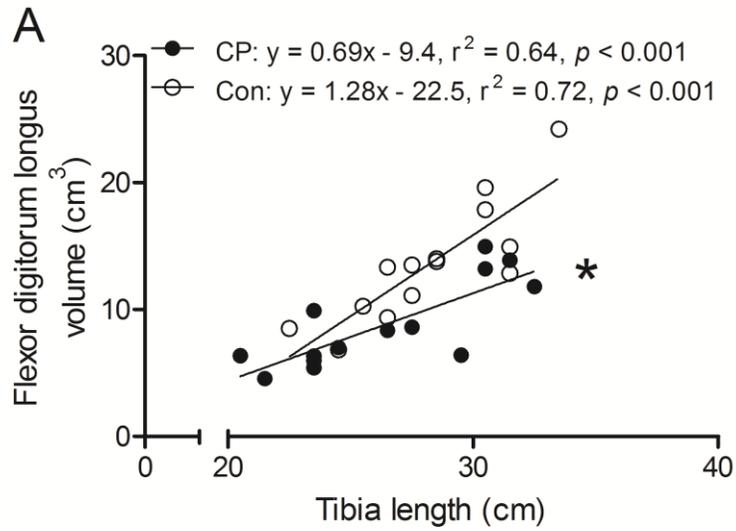


Figure 4.2. Scatter plot demonstrating the group difference in the (A) relationship between tibia length and flexor digitorum longus volume and the (B) relationship between tibia length and tibialis anterior volume. \* indicates group differences in the slope,  $p < 0.05$ .

There were positive relationships between PF regressed on gastrocnemius and soleus volume and DF regressed on tibialis anterior volume ( $r^2 = 0.32$  to  $0.71$ , all  $p < 0.05$ ; **Figure 3A - C**). When intramuscular fat concentration was removed from muscle volume (**Eq. 1**), the relationships became slightly stronger ( $r^2 = 0.37$  to  $0.77$ , all  $p < 0.05$ ; **Figure 3D - F**). There were no group differences in slopes (all  $p > 0.05$ ) but there were differences in the y-intercept (all  $p < 0.05$ ) for all uncorrected and corrected muscle volume-strength relationships. When strength was normalized to muscle volume, compared to controls, children with CP had lower PFgas and PFgas-fat (59 and 56 %, respectively, both  $p < 0.01$ ), PFsol and PFsol-fat (59 and 53 %, respectively, both  $p < 0.01$ ) and DFta and DFta-fat (51 and 48 %, respectively, both  $p < 0.01$ ) (**Figure 4**).

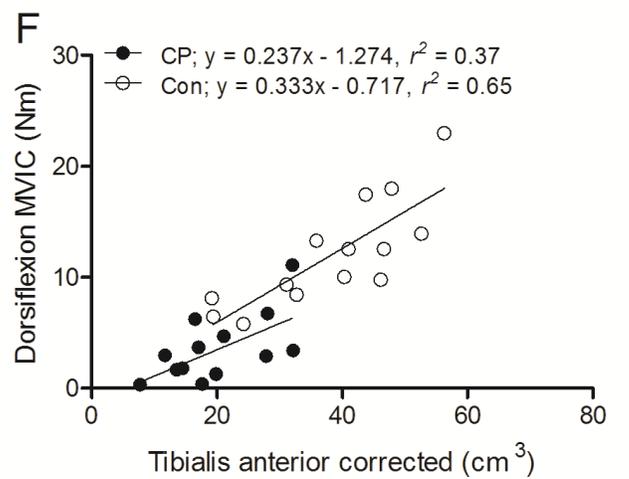
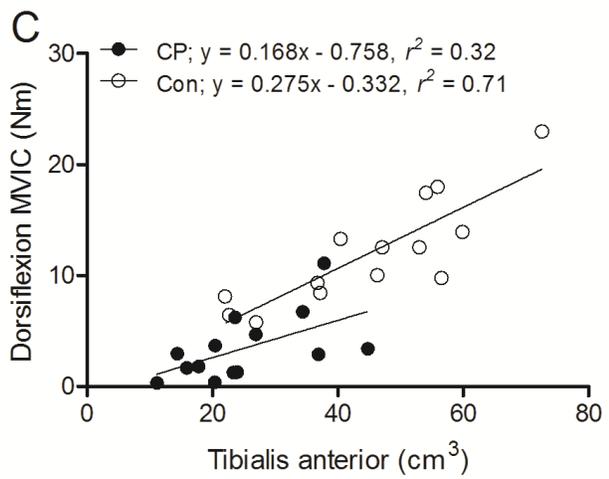
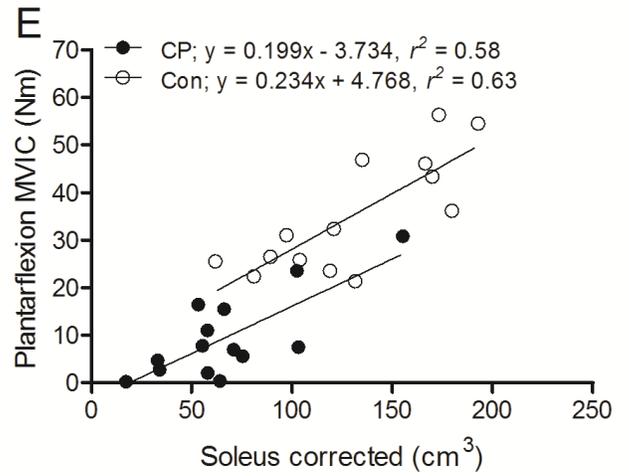
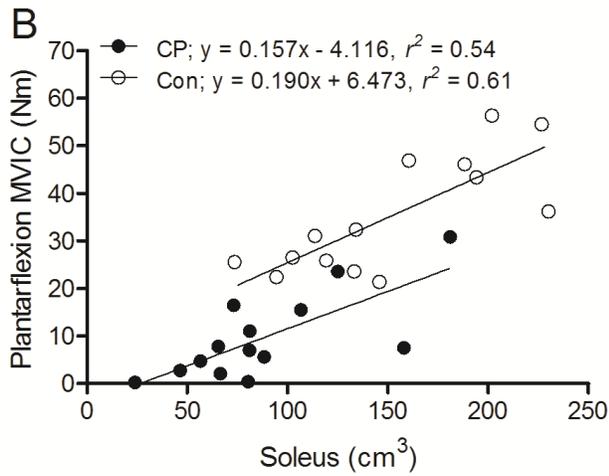
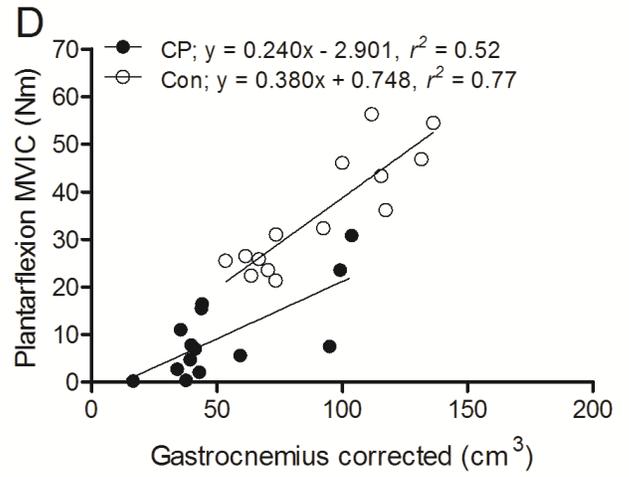
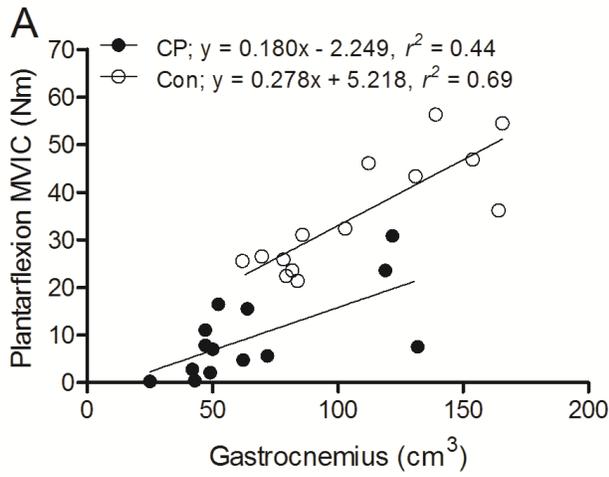


Figure 4.3. Scatter plot demonstrating the relationship between plantar flexor force and (A) gastrocnemius volume and (B) soleus volume and between dorsi flexor force and (C) tibialis anterior volume without correcting for intramuscular fat, and the relationship between plantar flexor force and (D) gastrocnemius volume and (E) soleus volume and between dorsi flexor force and (F) tibialis anterior volume after correcting for intramuscular fat in children with cerebral palsy (CP) and typically developing children (con). Muscle volumes were corrected intramuscular fat concentration using the following formula: Corrected muscle volume (cm<sup>3</sup>) = muscle volume (cm<sup>3</sup>) – [muscle volume (cm<sup>3</sup>) x fat concentration (%)]. Difference in the y-intercept for all relationships and all correlations are significant, *p* < 0.05.

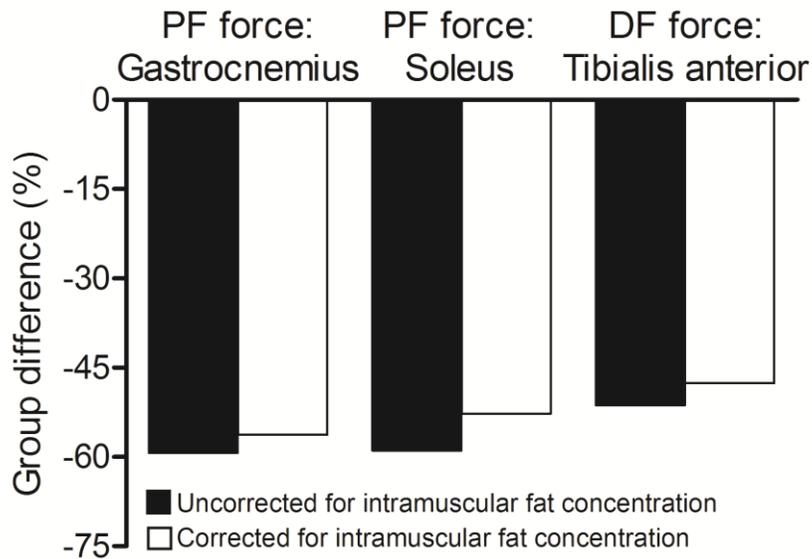


Figure 4.4. Bar graph shows the group differences (%) between the ratio of strength to muscle volume without correcting for intramuscular fat concentration (black bars) and after correcting for intramuscular fat concentration (open bars) for the gastrocnemius, soleus and tibialis anterior. PF, plantar flexor; DF, dorsi flexor. Muscle volumes were corrected intramuscular fat concentration using the following formula: Corrected muscle volume (cm<sup>3</sup>) = muscle volume (cm<sup>3</sup>) – [muscle volume (cm<sup>3</sup>) x fat concentration (%)].

## 4.5 Discussion

To our knowledge, this is the first study to assess the volume and fat concentration of individual leg muscles and their relation with muscle strength of the ankle in ambulatory children with spastic CP. Results from this study suggest that, compared to typically developing children, ambulatory children with spastic CP have a high concentration of fat in all muscles in the leg, which partially explains their weakness. This is concerning given the importance of muscle strength on motor function in children with CP and may give insight into potential mechanisms of the decline in mobility as children with CP age into their adult years [5].

The finding that ambulatory children with spastic CP have individual muscle volume deficits of the leg, ranging from 27 % to 44 %, is consistent with a previous study in a small group of older children with CP (11-17 y) [11]. In the current study, there were strong positive relationships between individual muscle volumes and tibia length in children with CP and controls. However, children with CP had lower muscle volume per given tibia length. The lack of difference in the slope of the regression lines for all but two muscles suggests that the muscle volume deficit in children with CP is consistent across tibia length. The lower slope for the flexor digitorum longus and tibialis anterior volume when regressed against tibia length suggests the deficit becomes more pronounced with increasing tibia length. This novel finding suggests that the growth of the flexor digitorum longus and tibialis anterior is more restricted than other leg muscles in children with CP; although, this interpretation should be taken cautiously given the limitations of cross-sectional observations. Nevertheless, the smaller tibialis anterior volume (44 %) and lower DF strength (71 %) in children with CP compared to typically developing children observed in this study may help explain why equinus deformity and toe drag are common gait complications in children with CP [12]. Bland

et al. [13] has demonstrated that tibialis anterior muscle thickness was positively associated with fast gait velocity and ankle kinematics during ambulation in children with hemiplegic spastic CP. Previous randomized control trials of muscle strength training in children and adolescents [14, 15] with CP have attempted to remedy the mobility- and gait-related complications of this population. While some studies showed improvement in measures of strength of the lower extremities [14, 16], these studies did not report on measures of DF or tibialis anterior parameters and may not have targeted the DFs directly. More recently, Kirk et al. [17] found that a 12-week explosive resistance training program improved DF rate of force development which was correlated with improvements of ankle gait kinematics in adults with CP. Therefore, interventions focused on increasing DF function and tibialis anterior volume may prove to be functionally important in children with CP.

Consistent with the literature [1, 2], we found that children with CP had substantially lower PF and DF strength and weaker muscle size-strength relationships than controls. In this study, we report that for a given muscle volume, children with CP have lower strength of the ankle. Therefore, the smaller muscle size does not fully explain the weaker strength of the ankle which is not entirely unexpected. Many factors contribute to muscle weakness in this pediatric population. The initial damage or malformation of the developing brain, resulting in CP, often disrupts the development and maturation [18] of the corticospinal tract which is largely responsible for motor control. The neuromuscular junction is also subject to disruptions [19] at the level of the neuromotor synapse [20]. Considering the altered signal transduction and nerve-muscle interface, it is not surprising that children with CP have dysfunctional neuromuscular activity of lower agonist and greater antagonist muscle activation during

a volitional maximal isometric contraction [21]. Although the etiology of CP is neural, muscle is plastic and is negatively affected by the disrupted neural input it receives. Compared to controls, DNA microarray analysis of the gracilis and semitendinosus muscles from children with spastic CP revealed lower metabolic capability and protein turnover and dramatic increases of extracellular matrix which was negatively associated with muscle fiber bundle stiffness [19]. Moreover, excessive fat infiltration of muscle has been shown to negatively associated with muscle fiber function [6] and contractile protein synthesis [22], thus contributing to muscle weakness and a blunted muscle response to muscle use (e.g., physical activity) and potentially restricting muscle growth. In the present study, children with CP had elevated fat concentration of all muscles of the leg. When muscle volume was corrected for intramuscular fat, the difference in muscle strength between children with CP and controls became less pronounced. This suggests that the elevated intramuscular fat infiltration contributes to muscle weakness in children with CP, albeit, to a small degree.

Intramuscular fat is a composite of intramyocellular (IMCL) and extramyocellular (EMCL) lipids with different etiologies and consequences in regards to energy metabolism and muscle function. To date, it is unknown if fat partitioning is favoring IMCL or EMCL in children with CP. Using MR spectroscopy in adults with neurological insults, Jonkers et al. [23] reported a 4.6-fold elevated EMCL and no difference in IMCL while Shah et al. [24] reported a 4.5-fold elevated EMCL and a 3.3-fold elevated IMCL compared to controls. The differences in the lipid deposition between intramuscular depots may be related to the difference in the time-since-injury and onset of physical inactivity between the studies ( $10.0 \pm 2.6$  vs.  $1.4 \pm 0.8$  years, respectively). These data infer a time-dependent adaptive mechanism of fatty acid

partitioning favoring EMCL. This is because IMCL serves as an immediate energy reservoir to facilitate myocyte contraction. Chronic physical inactivity, due to a neurological insult, would result in a chronically lower energetic demand from the muscle, and therefore, less reliance on IMCL. This is supported by evidence of physical inactivity downregulating proteins that are responsible for sequestering fatty acids towards IMCL, despite increases in intramuscular fat [25], suggesting a partitioning of fatty acids towards EMCL. However, these data are taken from adults [23-25] or adults who acquired a neurological insult at near- or full-development [23, 24]. Those with CP acquire a neurological insult around the time of birth. How low levels of physical activity and sedentary behavior throughout growth and development relate to molecular mechanisms of intramuscular fat partitioning is unknown.

Transcriptional analysis of spastic muscle from children with CP may indicate muscle damage [19]. Muscle damage is associated with an expansion and proliferation of fibro-adipogenic precursor cells that give rise to adipocytes. This process occurs at the expense of myocytes [26] and within the extramyocellular space [27]. The unformed myocytes in a muscle-damaged environment is thought to be a result from muscle precursor cells favoring a fibro-adipogenic progeny that would have otherwise given rise to satellite cells [26]. Satellite cells are primarily responsible for the development and regeneration of myocytes and has been found to be lower in children with CP [28]. Therefore, if there is a lesser population of satellite cells accompanied by a muscle damaged environment in children with CP, there may be a greater shift towards a fibro-adipogenic progeny. This may help to explain the inadequate accretion of muscle and allowing speculation that elevated intramuscular fat, observed in the present study, is driven by elevated EMCL. Whether the elevated intramuscular fat is due to elevated

EMCL, IMCL or a combination in children with CP is currently unknown. Knowing this information will help to better understand the pathophysiology of impaired muscle function and could provide insight into the health of the musculature.

In addition to the current study not distinguishing between IMCL and EMCL, another limitation is the small sample size. Therefore, the results should be interpreted with caution. Studies of children with CP using MRI are challenging because the children often have difficulty holding still due to spasticity. Moreover, the cost of MRI studies limits the number of participants that can be enrolled. However, despite the limited sample size, significant differences in muscle volume and fat concentration of individual leg muscles and relationships between muscle parameters and ankle strength were observed. This is attributed to the magnitude of the differences as well as the robustness of the measures. A strength of the study was that the children with CP were compared to a group of typically developing children not different in sex or age. Furthermore, the typically developing children were not different from the 50th sex- and age-based percentiles for height, body mass and BMI.

In conclusion, results from this study suggest that ambulatory children with a mild form of spastic CP have small individual leg muscles that are highly infiltrated with fat. Moreover, the results suggest that intramuscular fat contributes to muscle weakness in children with CP. Future studies are needed to determine if interventions focused on improving the size and function of the leg muscles, especially the tibialis anterior, will correspond to improvements in muscle function, mobility and health outcomes in this population. Future studies are also needed to determine if the intramuscular fat accretion is favoring EMCL or IMCL depots, which will help us better

understand the functional and health complications associated with the defective musculature in children with CP.

#### **4.6 Acknowledgements**

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## Chapter 5

### **GREATER CENTRAL ADIPOSITY IN AMBULATORY CHILDREN WITH SPASTIC CEREBRAL PALSY COMPARED TO TYPICALLY DEVELOPING CHILDREN**

#### **5.1 Abstract**

**Aims/hypothesis:** Children with cerebral palsy (CP) have low levels of physical activity and a high infiltration of skeletal muscle and bone marrow fat compared to typically developing children. This predisposes children with CP to an array of diseases of the musculoskeletal and cardiometabolic origin. The purpose of this study was to determine if ambulatory children with spastic CP have higher abdominal fat compared to typically developing children.

**Methods:** Eighteen ambulatory children with spastic CP (age,  $8.6 \pm 2.9$ ;  $n = 5$  girls) and 18 typically developing children matched to children with CP for age and sex participated in this study. Dual-energy x-ray absorptiometry was used to assess body composition and abdominal fat distribution.

**Results:** Compared to controls, children with CP had lower height percentile, total physical activity counts, spent more time in sedentary behavior and less time in moderate/vigorous physical activity (all  $p < 0.05$ ). There were no group differences in total body or trunk fat mass, total body or trunk fat-free mass, total body fat mass index (FMI), total body or trunk fat-free mass index (FFMI), abdominal, visceral and subcutaneous fat mass or subcutaneous FMI (all  $p > 0.05$ ). Compared to controls, children with CP had higher trunk, abdominal and visceral FMI (all  $p < 0.05$ ). There

were moderate relationships between visceral FMI and total physical activity counts and time spent in sedentary behavior ( $r^2 = 0.22$  and  $0.24$ , respectively, both  $p < 0.05$ ) for controls, but not for children with CP.

**Conclusions/interpretation:** There were no differences total body fat between groups. However, compared to typically developing children, ambulatory children with spastic CP had higher visceral fat which was not related to their lower levels of physical activity. These findings suggest a unique abdominal fat distribution pattern in children with CP. How it relates to cardiometabolic disease risk requires further investigation.

## 5.2 Introduction

Cerebral palsy (CP) results from damage or malformation of the developing brain causing impairments of motor function. Children with CP have low muscle strength [1], poor cardiorespiratory fitness [2] and low levels of physical activity [3]. These complications can lead to excess body fat accretion in childhood which is associated with increased cardiometabolic morbidity and mortality in adulthood [4-6].

Middle-aged adults with CP have a high multimorbidity prevalence, primarily consisting of musculoskeletal and cardiometabolic diseases, which is associated with obesity status [7]. This may be partially explained by the observed mobility decline as children with CP age into their adult years [8] and higher abdominal fat accompanied by smaller and lower quality muscle and bone in adults with CP compared to controls [9]. These data highlight the potential impact of low physical activity and poor musculoskeletal health on obesity-related complications in those with CP. Importantly, excess abdominal fat accumulation is associated with cardiometabolic disease risk factors, independent of total body fat, in the general population of children [10]. Children with CP often have a similar body mass index (BMI), a proxy for total body

fat, as typically developing children, but have higher musculoskeletal fat infiltration [3] suggesting a unique fat distribution pattern which is not captured by BMI. To date, the abdominal fat distribution profile in children with CP is unknown. The primary objective of this study was to determine if ambulatory children with spastic CP have higher abdominal fat compared to age- and sex-matched typically developing children. We hypothesized that ambulatory children with spastic CP vs. typically developing children would have higher visceral and subcutaneous fat mass.

### **5.3 Methods**

#### **5.3.1 *Study Participants***

Eighteen ambulatory children with mild spastic CP and 19 typically developing children (controls) that were between 4 and 12 years of age were included in this cross-sectional study. Of the 19 controls, 18 were matched to children with CP for age and sex. The Institutional Review Boards at the University of Delaware and AI duPont Hospital for Children approved the study procedures. Prior to testing, written consent and assent was obtained by the parents and the participants, respectively.

#### **5.3.2 *Anthropometry and Tanner Staging***

Height and body mass were measured while the child was in a t-shirt and shorts. Height was measured to the nearest 0.1 cm using a stadiometer (Seca 217; Seca GmbH & Co. KG., Hamburg, GER). Body mass was measured to the nearest 0.2 kg using a digital scale (Detecto, 6550, Cardinal Scale, Webb City, MO). Normative graphs published by the Centers for Disease Control and Prevention [11] were used to determine age- and sex-based percentiles of height, body mass and body mass index (BMI). Sexual maturity was assessed by a physician assistant using the Tanner staging

technique [12]. The technique is based on a 5-point scale, with I indicating no development and V indicating full development. Pubic hair and breast development were assessed in girls. Pubic hair and testicular/penile development were assessed in boys.

### **5.3.3** *Gross Motor Function Classification System (GMFCS)*

A physician assistant determined the gross motor function of children with CP using GMFCS [13]. This scale ranges from I to V with I and II reflecting gross motor independence, such as walking and running, but with limited ability of speed, balance and coordination, III reflecting the use of assistive walking devices and IV and V reflecting wheelchair empowered mobility.

### **5.3.4** *Physical Activity*

Physical activity was estimated using accelerometer-based activity monitors (Actical; Respironics Inc., Bend, OR). The activity monitors contain an omnidirectional accelerometer that is most sensitive to movements in the vertical plane when worn on the ankle and is sensitive to movements in the 0.5 to 3 Hz frequency range [14]. Physical activity counts were registered in 15 second epochs. Each participant wore two monitors on the lateral aspect of the ankle on the more affected side in children with CP and on the nondominant side in controls. Monitors were worn continuously (i.e., 24 hours per day) for four days (three week days and one weekend day). Participants and participant parents were instructed to take the monitors off only when swimming at a depth greater than 0.91 meters and during bathing/showering. This was confirmed by reviewing activity logs kept by the children with assistance from their parent and by visually examining the graphical output generated using software provided by the

manufacturer. If participants did not wear the monitors on any of the days, they were asked to re-wear the monitors to make up for the lost day(s). The total physical activity counts per day, time spent in sedentary, light and moderate/vigorous physical activity averaged from the two monitors are reported. The reliability of the total physical activity counts was assessed in 8 ambulatory children with mild CP and 8 typically developing children between 4 and 11 years of age who wore the monitors for four days on two separate occasions approximately one month apart. The intraclass correlation was 0.935 for children with CP and 0.913 for typically developing children indicating excellent reliability [3].

### **5.3.5** *Dual-energy X-ray Absorptiometry (DXA)*

Whole body DXA scans were acquired using standard imaging and positioning protocols (Discovery W, Pediatric Whole Body Analysis; Hologic Inc., Bedford, MA), software version 12.7.3.1. To limit motion during the scan, children with CP were secured from the waist down using the BodyFIX (Medical Intelligence Inc, Schwabmunchen, Germany) and a modified procedure, as previously described [15]. The modified BodyFIX procedure has no effect on body composition estimates from DXA in children [16]. Abdominal fat mass was obtained based on the manufacturer's instructions. Briefly, the android fat region was determined by an automatically defined region of interest box that was placed just above the iliac crest with a height that was set to 20 % of the height from the top of the iliac crest to the base of the skull. Abdominal fat was estimated within the android region based on the manufacturer's automated software. Visceral fat mass was estimated within the visceral cavity. Subcutaneous fat mass was estimated by subtracting visceral fat mass from total abdominal fat mass.

After completion of the scan, to account for differences in body height, fat mass index (FMI) and fat-free mass index (FFMI) from the total body (excluding the head), trunk and abdominal regions were determined by dividing tissue mass (kg) by height (m) squared as follows;

$$\text{FMI} = \text{fat mass (kg)} / \text{height (m)}^2$$

$$\text{FFMI} = \text{fat-free mass (kg)} / \text{height (m)}^2$$

### **5.3.6** *Statistics*

Data were analyzed using SPSS version 24.0 (IBM Corp, Armonk, NY). All variables were checked for normality by examining skewness and kurtosis. Group differences between children with CP and controls were determined using an independent t test if the data were normally distributed and a Mann-Whitney U test if the data were non-normally distributed. Bivariate linear regression was used to determine the relationship between visceral FMI and measures of physical activity and between subcutaneous FMI and measures of physical activity. Group interactions were assessed. There were an equal number of boys and girls in each group. Due to the small number of girls included in the study ( $n = 5/\text{group}$ ), sex differences were not considered in the statistical analysis. Values are presented as mean  $\pm$  SD unless stated otherwise. The magnitude of the effects were determined using Cohen's  $d$  ( $d = \text{mean difference between groups} / \text{pooled SD}$ ), with 0.2, 0.5 and 0.8 representing small, moderate and large effect sizes, respectively [17].

## **5.4 Results**

Physical characteristics and physical activity of the 18 age- and sex-matched pairs included in the study are presented in **Table 1**. Compared to controls, children

with CP had lower height percentile ( $d = 1.242, p < 0.001$ ) but there were no differences in age, Tanner stage pubic hair or testicular-penile/breast, body mass, body mass percentile, BMI or BMI percentile (all  $d < 0.66, p > 0.05$ ). Although marginally insignificant ( $d = 0.675, p = 0.054$ ), children with CP had lower height compared to controls. Compared to controls, children with CP had 56 % fewer total physical activity counts at the ankle ( $d = 2.489, p < 0.001$ ), spent more time in sedentary behavior ( $d = 1.108, p = 0.002$ ) and less time in moderate/vigorous activity ( $d = 1.315, p < 0.001$ ).

Table 5.1. Characteristics of children with cerebral palsy (CP) and typically developing children (con).

|                          | CP (n = 18)       | Con (n = 18)      | <i>p</i>         | <i>d</i> |
|--------------------------|-------------------|-------------------|------------------|----------|
| <b>Characteristics</b>   |                   |                   |                  |          |
| Age (y)                  | 8.6 ± 2.9         | 8.9 ± 2.1         | 0.654            | 0.153    |
| Sex female, n (%)        | 5 (28 %)          | 5 (28 %)          |                  |          |
| Tanner stage (I/II/III)  |                   |                   |                  |          |
| Pubic hair               | 12/5/1            | 13/3/2            | 0.445            |          |
| Testicular-penile/breast | 15/2/1            | 13/4/1            | 0.788            |          |
| Height (m)               | 1.23 ± 0.16       | 1.33 ± 0.12       | 0.054            | 0.675    |
| Height (%)               | 19 ± 23           | 51 ± 29           | <b>&lt;0.001</b> | 1.242    |
| Body mass (kg)           | 28.2 ± 11.4       | 29.4 ± 6.4        | 0.694            | 0.138    |
| Body mass (%)            | 36 ± 34           | 50 ± 29           | 0.179            | 0.459    |
| BMI (kg/m <sup>2</sup> ) | 17.9 ± 4.4        | 16.6 ± 2.2        | 0.562            | 0.387    |
| BMI (%)                  | 55 ± 34           | 47 ± 31           | 0.487            | 0.235    |
| GMFCS (I/II)             | 7/11              | -                 |                  |          |
| <b>Physical activity</b> |                   |                   |                  |          |
| Total (counts/day)       | 299,081 ± 123,589 | 680,052 ± 182,517 | <b>&lt;0.001</b> | 2.489    |
| Sedentary (%)            | 65.9 ± 7.5        | 58.1 ± 6.6        | <b>0.002</b>     | 1.108    |
| Light (%)                | 29.7 ± 7.7        | 33.3 ± 5.0        | 0.107            | 0.565    |
| Moderate/vigorous (%)    | 4.4 ± 3.7         | 8.6 ± 2.7         | <b>&lt;0.001</b> | 1.315    |

BMI, body mass index; GMFCS, gross motor function classification system. Differences between children with CP and Con were determined using independent *t*

test or Mann Whitney *U* test. Values are means  $\pm$  SD. *d* = the effect size of group differences. Significant differences are bolded.

Group comparisons of body composition measures of the total body and trunk are presented in **Table 2**. There were no group differences for total body fat mass or fat-free mass, trunk fat mass or fat-free mass, total body FMI or FFMI or trunk FFMI (all  $d < 0.48$ ,  $p > 0.05$ ). Compared to controls, children with CP had higher trunk FMI ( $d = 0.512$ ,  $p = 0.019$ ).

Table 5.2. Body composition of children with cerebral palsy (CP) and typically developing children (con).

|                                      | CP (n = 18)    | Con (n = 18)   | <i>p</i>     | <i>d</i> |
|--------------------------------------|----------------|----------------|--------------|----------|
| Total body fat (kg)                  | 8.3 $\pm$ 5.2  | 7.2 $\pm$ 3.1  | 0.474        | 0.250    |
| Total body fat-free (kg)             | 16.1 $\pm$ 6.5 | 18.6 $\pm$ 4.0 | 0.189        | 0.461    |
| Trunk fat (kg)                       | 3.5 $\pm$ 2.4  | 2.9 $\pm$ 1.4  | 0.606        | 0.316    |
| Trunk fat-free (kg)                  | 8.7 $\pm$ 3.3  | 9.7 $\pm$ 1.9  | 0.267        | 0.391    |
| Total body FMI (kg/m <sup>2</sup> )  | 5.2 $\pm$ 2.7  | 4.1 $\pm$ 1.7  | 0.133        | 0.478    |
| Total body FFMI (kg/m <sup>2</sup> ) | 10.2 $\pm$ 2.0 | 10.4 $\pm$ 0.9 | 0.151        | 0.169    |
| Trunk FMI (kg/m <sup>2</sup> )       | 2.2 $\pm$ 1.3  | 1.7 $\pm$ 0.8  | <b>0.019</b> | 0.512    |
| Trunk FFMI (kg/m <sup>2</sup> )      | 5.5 $\pm$ 1.0  | 5.5 $\pm$ 0.4  | 0.847        | 0.067    |

FMI, fat mass index; FFMI, fat-free mass index. Differences between children with CP and Con were determined using independent *t* test or Mann Whitney *U* test. Values are means  $\pm$  SD. *d* = the effect size of group differences. Significant differences are bolded.

Group comparisons of body composition measures of abdominal, visceral and subcutaneous regions are shown in **Figure 1**. There were no group differences for abdominal fat mass ( $d = 0.449$ ,  $p = 0.217$ ; **Figure 1A**), visceral fat mass ( $d = 0.508$ ,  $p = 0.193$ ; **Figure 1B**) or subcutaneous fat mass ( $d = 0.312$ ,  $p = 0.193$ ; **Figure 1C**). Compared to controls, children with CP had higher abdominal FMI ( $d = 0.587$ ,  $p =$

0.002; **Figure 1D**) and visceral FMI ( $d = 0.670$ ,  $p = 0.001$ ; **Figure 1E**). Although marginally insignificant ( $d = 0.330$ ,  $p = 0.088$ ), children with CP had higher subcutaneous FMI compared to controls (**Figure 1F**).

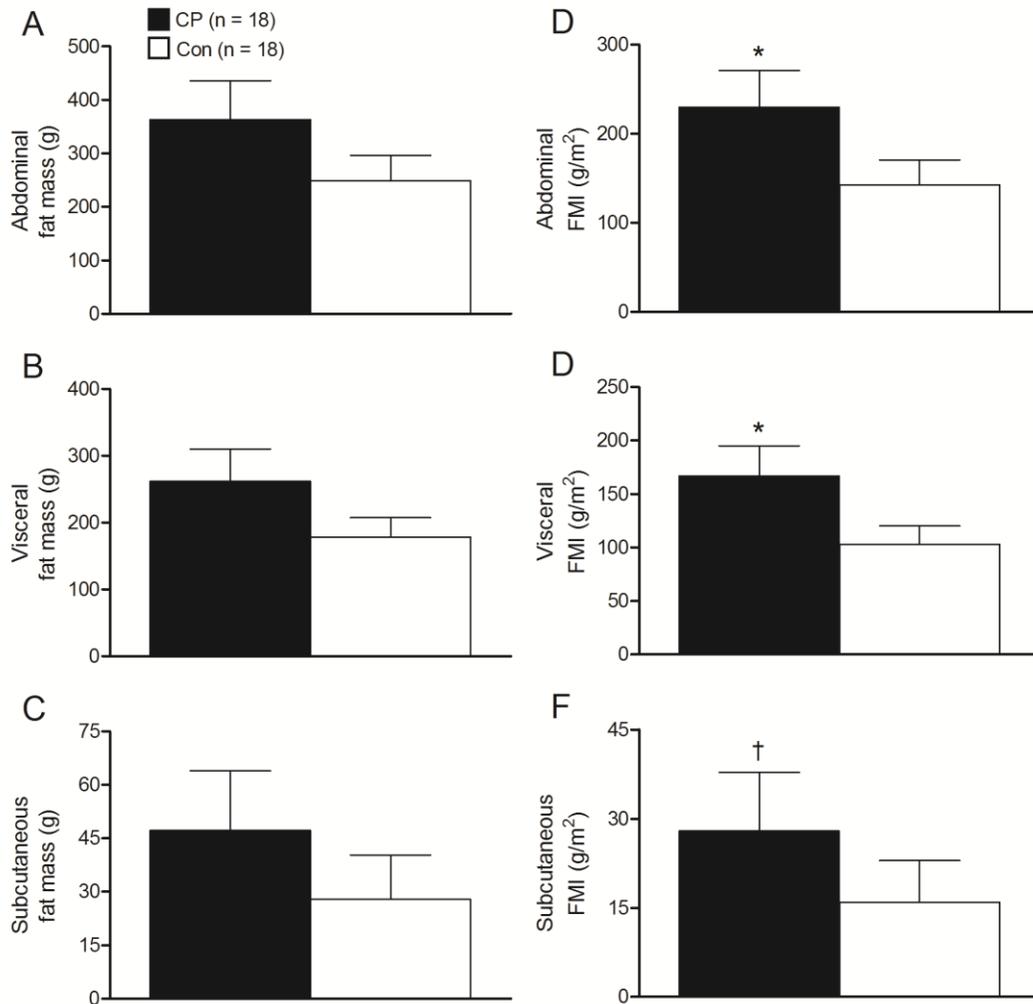


Figure 5.1. Bar graphs represent fat mass of (A) abdominal, (B) visceral and (C) subcutaneous regions and fat mass index (FMI) of (D) abdominal, (E) visceral and (F) subcutaneous regions for ambulatory children with spastic cerebral palsy (CP; black bar) and typically developing children (Con; white bar). \*Different from controls,  $p < 0.05$ . †Different from controls,  $p = 0.088$ .

Scatter plots demonstrate the relationships between visceral FMI (**Figure 2**) or subcutaneous FMI (**Figure 3**) with total physical activity counts and time spent in sedentary, light and moderate/vigorous physical activity. Visceral FMI or subcutaneous FMI were not related to any of the measures of physical activity in children with CP (all  $r^2 < 0.03$ ,  $p > 0.05$ ). For controls, there were moderate relationships between visceral FMI and total physical activity counts ( $r^2 = 0.22$ ,  $p = 0.050$ ) and time spent in sedentary behavior ( $r^2 = 0.24$ ,  $p = 0.039$ ). For controls, visceral FMI was not related to time spent in light or moderate/vigorous physical activity and subcutaneous FMI was not related to any of the measures of physical activity (all  $r^2 < 0.20$ ,  $p > 0.05$ ).

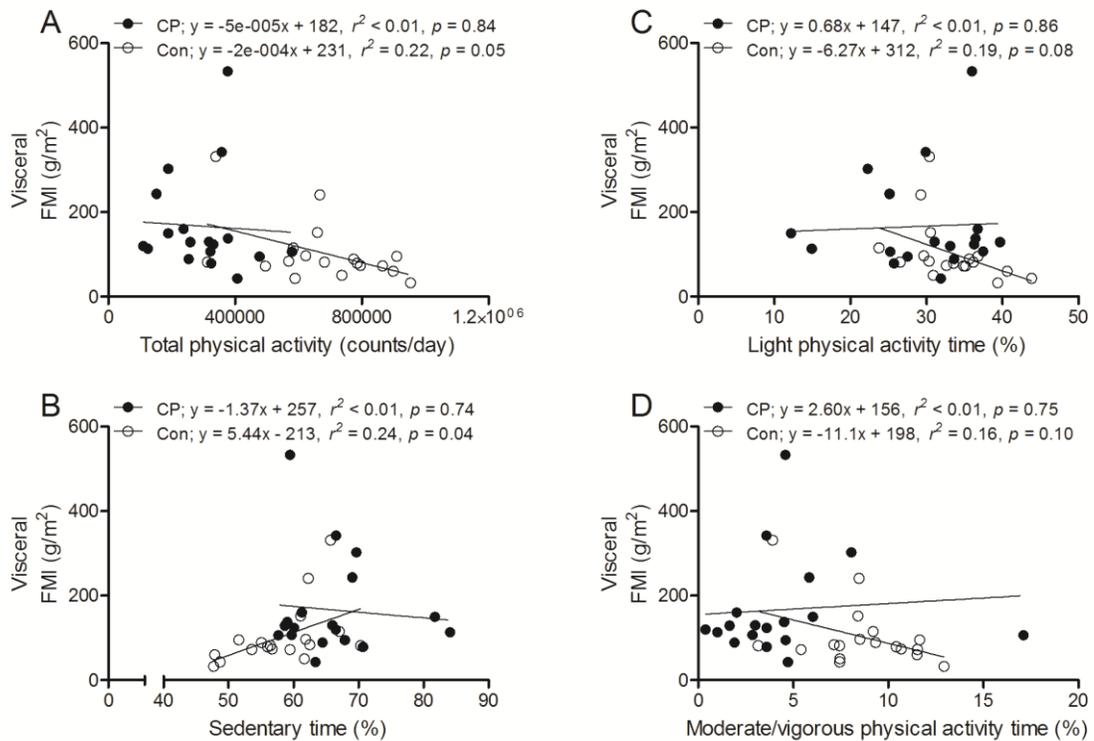


Figure 5.2. Scatter plot demonstrating the relationships between visceral fat mass index (FMI) and (A) total physical activity and time spent in (B) sedentary, (C) light and (D) moderate/vigorous physical activity for ambulatory children with spastic cerebral palsy (CP; closed circles) and typically developing children (Con; open circles).

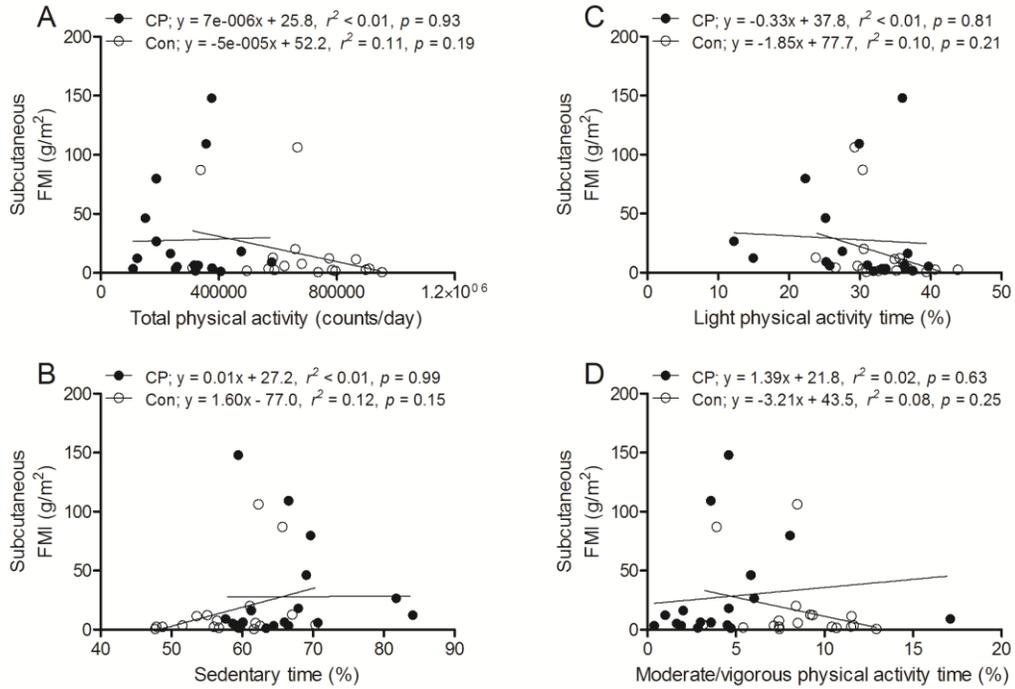


Figure 5.3. Scatter plot demonstrating the relationships between subcutaneous fat mass index (FMI) and (A) total physical activity and time spent in (B) sedentary, (C) light and (D) moderate/vigorous physical activity for ambulatory children with spastic cerebral palsy (CP; closed circles) and typically developing children (Con; open circles).

## 5.5 Discussion

To our knowledge, this is the first study to report greater central adiposity measures including trunk, abdominal and visceral fat in ambulatory children with spastic CP compared to typically developing children. This is particularly important

because adults with CP present with a high prevalence of multimorbidity, especially the coupling of musculoskeletal and cardiovascular diseases, which is associated with obesity status [7]. Further, different fat depots, rather than total body fat [10], may play a unique role on central, hepatic and peripheral glucose regulation. Therefore, identifying abnormal obesity indices in children with CP is crucial. This study provides insight at an early stage of development for the necessity of interventions aimed towards mitigating the development of obesity-related complications in those with CP.

The finding that ambulatory children with spastic CP have higher visceral fat is consistent with a study in adults. Peterson et al. [9] reported that adults with CP had greater visceral fat area and after controlling for age, sex and body mass, subcutaneous fat area at the level of the 4th lumbar vertebrae was also higher than controls. However, this population tends to be shorter and present with musculoskeletal deficits compared to their typically developing peers [3]. Therefore, reporting absolute values, such as areas and volumes, may not be appropriate for those with CP. In the current study, we found no differences in the absolute mass of any of the total body, trunk or abdominal fat depots between children with CP and typically developing children. However, children with CP had higher trunk and visceral FMI.

The finding that ambulatory children with CP had lower levels of physical activity is consistent with other studies [3, 18]. In the current study, we found that visceral FMI was positively related to sedentary behavior and negatively related to total physical activity in typically developing children but not in children with CP. Moreover, subcutaneous FMI was not related to any measures of physical activity in either group, which is consistent with a study in typically developing children [19]. The lack of relationships between measures of physical activity and visceral FMI suggests

other factors may be responsible for the excess accrual of fat within the visceral region in children with CP. Using magnetic resonance imaging at the level of the middle third thigh, Johnson et al. [18] reported negative relationships between physical activity levels and intermuscular ( $r = -0.76, p < 0.01$ ) and subfascial ( $r = -0.63, p = 0.03$ ) adipose tissue in nonambulatory children with CP. It is possible that peripheral fat depots (e.g., skeletal muscle) may be more sensitive to low levels of physical activity than abdominal or total body depots in children with CP.

The present study has several strengths. First, we used DXA to assess body composition and abdominal fat distribution in children with CP. DXA provides many measures of body composition with just one or a few scans, has relatively fast scanning times and does not require labor-intensive analysis procedures like computed tomography (CT) or magnetic resonance imaging. This is important working with this population because of the involuntary spasms and difficult time holding still which can limit total scanning time and negatively affect image quality. Second, DXA is measuring tissue distribution within a region of the abdomen rather than at a single slice, which is widely used with in vivo imaging of abdominal fat distribution [9, 20-22]. Research has shown inconsistencies with a single slice in predicting volumetric regions of visceral and subcutaneous fat [23-25] and their relationships with cardiometabolic disease risk factors [23, 25]. These differences are likely due to variations of tissue distribution within the abdomen. Therefore, larger regions, rather than a single slice, are more representative of tissue distribution and are more sensitive to detect differences in younger, smaller or leaner children [26] and in populations with a unique body composition, such as children with CP.

The limitations of this study must also be discussed. The region where visceral and subcutaneous fat mass were obtained may have included the inferior part of the liver. Higher liver fat has been reported in populations with higher abdominal fat distributions, such as obese adolescents [27]. Using the DXA method in the present study from a whole body scan, it is not possible to segment out the liver, such as in other in vivo imaging techniques. Furthermore, it is unknown if children with CP have elevated fat infiltration of the liver. Although, capturing a small portion of liver fat would not likely influence the entire visceral fat measure to a significant extent due to its small volumetric contribution to that region of interest. Lastly, markers of cardiometabolic health were not assessed. It is unknown if the higher visceral fat in ambulatory children with CP is associated with a higher risk for cardiometabolic diseases.

In conclusion, compared to typically developing children, ambulatory children with spastic CP have higher visceral fat. These data demonstrate the elevated risk for accelerating the development of obesity-related complications, seen in adults with CP [7], before reaching puberty. Future studies are needed to track the development of obesity indices throughout growth and development and to identify effective treatment strategies to limit or negate the excess accumulation of fat within the visceral cavity in children with CP.

## **5.6 Acknowledgements**

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## Chapter 6

### **BMI DOES NOT CAPTURE THE HIGH FAT MASS INDEX AND LOW FAT-FREE MASS INDEX IN CHILDREN WITH CEREBRAL PALSY AND PROPOSED STATISTICAL MODELS THAT IMPROVE THIS ACCURACY**

#### **6.1 Abstract**

**BACKGROUND:** Children with cerebral palsy (CP) are at risk for having a misclassified overweight/obesity status based on BMI thresholds due to their lower fat-free mass and similar fat mass compared to typically developing children.

**OBJECTIVES:** The primary objective was to determine if BMI could predict fat mass index (FMI) and fat-free mass index (FFMI) in children with CP.

**DESIGN:** Forty-two children with CP and 42 typically developing children matched to children with CP for age and sex participated in the study. Dual-energy x-ray absorptiometry was used to assess body composition. Children with CP who could ambulate without assistance were considered ambulatory (ACP) and the rest were considered nonambulatory (NACP).

**RESULTS:** Children with CP had higher percent body fat (%Fat) and FMI and lower fat-free mass and FFMI than controls ( $p < 0.05$ ) but no difference in fat mass ( $p = 0.10$ ). When BMI was statistically controlled, NACP had higher %Fat, fat mass and FMI and lower FFMI than ACP and controls ( $p < 0.05$ ). NACP had lower fat-free mass than controls ( $p < 0.05$ ). ACP had higher %Fat and FMI and lower fat-free mass and FFMI than controls ( $p < 0.05$ ). BMI was a strong predictor of FMI ( $r^2 = 0.83$ ) and a moderately-strong predictor of FFMI ( $r^2 = 0.49$ ) in children with CP (both  $p < 0.01$ ).

Prediction of FMI ( $R^2 = 0.86$ ) and FFMI ( $R^2 = 0.66$ ) from BMI increased ( $p < 0.05$ ) when age, sex and ambulatory status were included.

**CONCLUSION:** Compared to typically developing children, children with CP have a higher FMI and lower FFMI for a given BMI which is more pronounced in NACP than ACP. The finding suggests that the prevalence of overweight/obesity status may be underestimated in children with CP.

## **6.2 Introduction**

Body composition is very important in the assessment of nutritional status because of the alarming rise in childhood obesity [1] and the strong relationship between the level of body fat and chronic disease risk [2] and cardiovascular mortality [3]. Body mass index (BMI) is commonly used to assess obesity status and is an accurate predictor of fat mass in typically developing children [4]. Unfortunately, BMI only serves as a proxy of total body fat because it does not distinguish between fat and fat-free components of the body. Percent body fat (%Fat), which is determined by dividing fat mass by total body mass, is a better marker of relative adiposity and disease risk than BMI because the fat and fat-free components of the body are measured. However, %Fat is limited because it may be driven by variations in fat mass, fat-free mass or both. Therefore, the use of BMI and %Fat in classifying overweight/obesity status is questionable in populations with altered body composition.

Children with cerebral palsy (CP) are at an increased risk for having a misclassified overweight/obesity status based on BMI and %Fat because of their lower fat-free mass [5-7], but similar fat mass [5, 8, 9] compared to typically developing children. BMI-based thresholds suggest the prevalence of obesity in children with mild-to-moderate CP [10] is similar to [1] or slightly lower than [11] the general population

of children and that ambulatory children with CP have a greater likelihood of being overweight/obese than children with CP with greater levels of motor impairment [10, 12]. It is possible that these findings are driven by the greater musculoskeletal deficits seen in nonambulatory than ambulatory children with CP [13-15] rather than lower accretion of fat. Therefore, BMI may not be capturing the true level of adiposity and leanness in children with CP, which may be further complicated by ambulatory status.

In an attempt to remedy the limitations of BMI and %Fat in the assessment of body composition in children [16], fat mass index (FMI) and fat-free mass index (FFMI) have been proposed [17-19]. The advantage of FMI and FFMI over BMI and %Fat is that they distinguish between fat and fat-free mass, are relative to the individual's height rather than body mass and they are not influenced by variation of fat-free mass or fat mass, respectively. However, to get an accurate assessment of FMI and FFMI, methods that are expensive, require extensive training and/or expose participants to ionizing radiation must be performed. The primary objective of the current study was to determine whether BMI can accurately estimate FMI and FFMI in children with CP. It was hypothesized that 1) BMI would correlate with FMI and FFMI but would underestimate FMI and overestimate FFMI in children with CP, and 2) these discrepancies would be more pronounced in nonambulatory than ambulatory children with CP. The secondary objective of this study was to create statistical models to estimate FMI and FFMI based on BMI and other easily obtained characteristics.

## **6.3 Subjects and Methods**

### **6.3.1 *Subjects and Study Design***

Forty-two children with CP and 73 typically developing children (controls), some of which had partaken in previous studies [15, 20-23], were included in this cross-sectional study. Of the 73 controls, 42 were matched to children with CP for age ( $\pm 1.5$  y), sex and race and were between the 5<sup>th</sup> and 95<sup>th</sup> percentile for height, body mass and BMI. All matched participants were between 4 and 12 years of age.

### **6.3.2 *Anthropometrics***

Height and body mass were measured while children wore minimal clothing and were without shoes or braces. For ambulatory children with CP and controls, height was measured in an erect standing position using a stadiometer to the nearest 0.1 cm. For nonambulatory children with CP, height was estimated from knee height using a caliper (Ross Knee Height Caliper, MedHelp, San Francisco, CA) and the equation by Stevenson et al. [24]. Height was also estimated using forearm length as described by Miller et al. [25]. Body mass of all children was determined using a digital scale (Detecto 6550, Cardinal Scale, Webb City, MO) to the nearest 0.1 kg. Normative graphs published by the Centers for Disease Control and Prevention [26] were used to determine age- and sex-based percentiles of height, body mass and BMI.

### **6.3.3 *Gross Motor Function Classification System (GMFCS)***

A physician assistant determined the gross motor function of children with CP using GMFCS [27]. This scale ranges from I to V with I and II reflecting gross motor independence, such as walking and running, but with limited ability of speed, balance and coordination, III reflecting the use of assistive walking devices and IV and V

reflecting wheelchair empowered mobility. In the current study, children with CP who could ambulate without an assistive device (i.e., GMFCS of I and II) were considered ambulatory and children with CP who could only ambulate with an assistive device (i.e., GMFCS III) or could not ambulate (i.e., GMFCS IV and V) were considered nonambulatory.

#### **6.3.4** *Dual-energy X-ray Absorptiometry (DXA)*

A total body scan was performed using DXA (Discovery W, Pediatric Whole Body Analysis; Hologic Inc., Bedford, MA). To limit motion during the scan, children with CP were secured from the waist down using the BodyFIX (Medical Intelligence Inc, Schwabmunchen, Germany) and a modified procedure, as previously described [21]. The modified BodyFIX procedure has no effect on body composition estimates from DXA in children [20].

After completion of the scan, total body (excluding the head) FMI and FFMI were determined by dividing tissue mass (kg) by height (m) squared as follows;

$$\text{FMI} = \text{fat mass (kg)} / \text{height (m)}^2$$

$$\text{FFMI} = \text{fat-free mass (kg)} / \text{height (m)}^2$$

#### **6.3.5** *Ethics*

This study was conducted according to the guidelines of the Declaration of Helsinki of 1975 as revised in 1983. The study was approved by the Institutional Review Boards at AI duPont Hospital for Children, Wilmington DE and the University of Delaware, Newark DE. Consent and assent were obtained from parents/guardians and participants, respectively.

### 6.3.6 *Statistical Analysis*

Data were analyzed using SPSS version 24.0 (IBM Corp, Armonk, NY). Height, height percentile, BMI, FMI and FFMI were determined using standing height for ambulatory children with CP and controls and height estimated from knee height in nonambulatory children with CP. All variables were checked for normality by examining skewness and kurtosis. Group differences between children with CP and controls were determined using an independent t test if the data were normally distributed and a Mann-Whitney U test if the data were non-normally distributed. Subgroup differences among nonambulatory children with CP, ambulatory children with CP and controls were determined by ANOVA and ANCOVA using BMI as a covariate. Bonferroni post hoc tests were conducted if the variances were equal or Games-Howell post hoc tests if the variances were unequal. Values are presented as mean  $\pm$  SD unless stated otherwise. The magnitude of the effects were determined using Cohen's *d* (*d*), with 0.2, 0.5 and 0.8 representing small, moderate and large effect sizes, respectively [28].

Bivariate linear regression was used to determine the relationship between BMI and FMI and between BMI and FFMI. The interaction of BMI-FMI and BMI-FFMI was assessed between groups. Multiple linear regression was used to determine the amount of variance of FMI and FFMI explained by BMI, age, sex and a dichotomous variable for ambulatory status for children with CP (i.e., ambulatory or nonambulatory). All independent predictors (i.e., BMI, age, sex and ambulatory status) were examined for interactions and if they did not significantly contribute, they were removed from the final model. We performed the same set of analyses but using forearm length to estimate height for BMI, FMI and FFMI in nonambulatory children with CP. The rationale for this was that clinics that treat those with CP may only use knee height or forearm length

for nonambulatory children with CP. The resulting models using height estimated from knee height for nonambulatory children with CP were cross-validated in children with CP using the leave-one-out method [29].

## 6.4 Results

### 6.4.1 *Physical Characteristics of Study Participants*

The physical characteristics of children with CP and their 42 matched controls are shown in **Table 1**. Compared to controls, children with CP had lower height ( $d = 0.649, p = 0.004$ ), height percentile ( $d = 1.291, p < 0.001$ ) and body mass percentile ( $d = 0.820, p < 0.001$ ). There were no group differences in age ( $d = 0.054, p = 0.806$ ) or body mass ( $d = 0.350, p = 0.115$ ). When children with CP were separated based on ambulatory status, compared to controls, nonambulatory children with CP had lower height percentile ( $d = 1.484, p < 0.001$ ) and body mass percentile ( $d = 0.991, p = 0.011$ ). Compared to controls, ambulatory children with CP had lower height ( $d = 0.690, p = 0.019$ ), height percentile ( $d = 1.151, p < 0.001$ ) and body mass percentile ( $d = 0.688, p = 0.045$ ). There were no differences in physical characteristics between nonambulatory and ambulatory children with CP (all  $d < 0.58, p > 0.05$ ).

### 6.4.2 *BMI, BMI Percentile and Weight Classification*

There were no differences between all children with CP and controls or among nonambulatory and ambulatory children with CP and controls for BMI or BMI percentile (all  $d < 0.19, p > 0.05$ ). Furthermore, BMI percentile was not different from the 50<sup>th</sup> age- and sex-based percentile in all children with CP, nonambulatory and ambulatory children with CP and controls (all  $p > 0.05$ ). When children with CP and controls were separated based on BMI percentile into underweight ( $< 5^{\text{th}}$  percentile),

normal/healthy weight (5<sup>th</sup> to 85<sup>th</sup> percentile) or overweight/obese (> 85<sup>th</sup> percentile) categories, 23.8 % of the CP group (n = 10) were underweight, 59.5 % (n = 25) were normal/healthy weight and 16.7 % (n = 7) were overweight/obese. The control group had 1 child (2.4 %) that was underweight, 85.7 % (n = 36) were normal/healthy weight and 11.9 % (n = 5) were overweight/obese. When children with CP were separated based on ambulatory status, 33.3 % (n = 6) of the nonambulatory children were underweight, 50 % (n = 9) were normal/healthy weight and 16.7 % (n = 3) were overweight/obese while 16.7 % (n = 4) of the ambulatory children with CP were underweight, 66.7 % (n = 16) were normal/healthy weight and 16.7 % (n = 4) were overweight/obese.

Table 6.1. Physical characteristics of children with cerebral palsy (CP) and controls (con).

|                          | All CP (n = 42)          | NACP (n = 18)        | ACP (n = 24)             | Con (n = 42) |
|--------------------------|--------------------------|----------------------|--------------------------|--------------|
| Age (y)                  | 9.1 ± 2.5                | 9.9 ± 2.1            | 8.5 ± 2.6                | 9.2 ± 2.2    |
| Sex male, n (%)          | 25 (60 %)                | 7 (39 %)             | 18 (75 %)                | 25 (60 %)    |
| Height (m)               | 1.26 ± 0.16 <sup>1</sup> | 1.28 ± 0.16          | 1.25 ± 0.16 <sup>1</sup> | 1.35 ± 0.13  |
| Height (%)               | 23 ± 27 <sup>1</sup>     | 19 ± 27 <sup>1</sup> | 27 ± 28 <sup>1</sup>     | 56 ± 24      |
| Body mass (kg)           | 27.9 ± 10.9              | 28.3 ± 11.5          | 27.7 ± 10.7              | 31.3 ± 8.4   |
| Body mass (%)            | 31 ± 33 <sup>1</sup>     | 27 ± 33 <sup>1</sup> | 35 ± 33 <sup>1</sup>     | 53 ± 21      |
| BMI (kg/m <sup>2</sup> ) | 17.1 ± 3.9               | 17.2 ± 4.1           | 17.1 ± 3.9               | 16.8 ± 2.1   |
| BMI (%)                  | 47 ± 36                  | 44 ± 39              | 50 ± 34                  | 50 ± 28      |

NACP, nonambulatory CP; ACP, ambulatory CP. Values are means ± SD. % reflects the percentile relative to age- and sex-based norms. <sup>1</sup>Different compared to controls, *p* < 0.05.

#### 6.4.3 %Fat, Fat Mass and Fat-free Mass

Estimates of %Fat, fat mass and fat-free mass are shown in **Table 2**. Compared to controls, children with CP had higher %Fat ( $d = 0.838$ ,  $p < 0.001$ ) and lower fat-free mass ( $d = 0.729$ ,  $p = 0.001$ ), but no difference in fat mass ( $d = 0.387$ ,  $p = 0.102$ ). When BMI was statistically controlled, the differences between children with CP and controls remained and fat mass was higher in children with CP ( $p = 0.020$ ). When children with CP were separated based on ambulatory status, compared to controls, nonambulatory children with CP had higher %Fat ( $d = 1.144$ ,  $p = 0.005$ ) and lower fat-free mass ( $d = 0.881$ ,  $p = 0.011$ ). Compared to controls, ambulatory children with CP had lower fat-free mass ( $d = 0.620$ ,  $p = 0.045$ ). There were no differences in %Fat, fat mass or fat-free mass between nonambulatory and ambulatory children with CP (all  $d < 0.52$ ,  $p > 0.05$ ). When BMI was statistically controlled, compared to ambulatory children with CP and controls, nonambulatory children with CP had higher %Fat ( $p = 0.030$  and  $p < 0.001$ , respectively) and fat mass ( $p = 0.037$  and  $p = 0.002$ , respectively). Nonambulatory children also had lower fat-free mass than controls ( $p = 0.001$ ). Furthermore, compared to controls, ambulatory children with CP had higher %Fat ( $p = 0.041$ ) and lower fat-free mass ( $p = 0.006$ ).

#### 6.4.4 FMI and FFMI

Estimates of FMI and FFMI are shown in **Table 2**. Compared to controls, children with CP had higher FMI ( $d = 0.650$ ,  $p = 0.033$ ) and lower FFMI ( $d = 0.639$ ,  $p = 0.005$ ). When BMI was statistically controlled, the differences between children with CP and controls remained. When children with CP were separated based on ambulatory status, compared to controls, nonambulatory children with CP had higher FMI, although, marginally insignificant ( $d = 0.825$ ;  $p = 0.077$ ), and lower FFMI ( $d = 1.046$ ;

$p = 0.003$ ). There were no differences in FMI or FFMI between ambulatory children with CP and controls (both  $d < 0.51$ ,  $p > 0.05$ ) or between nonambulatory and ambulatory children with CP (both  $d < 0.55$ ,  $p > 0.05$ ). When BMI was statistically controlled, compared to controls, both nonambulatory and ambulatory children with CP had higher FMI ( $p < 0.001$  and  $0.019$ , respectively) and lower FFMI ( $p < 0.001$  and  $0.036$ , respectively). Compared to ambulatory children with CP, nonambulatory children with CP had higher FMI ( $p = 0.032$ ) and lower FFMI ( $p = 0.028$ ). **Figure 1** highlights the lack of group difference in BMI, but the unique distribution of FMI (nonambulatory children with CP > ambulatory children with CP > controls) and FFMI (nonambulatory children with CP < ambulatory children with CP < controls) when BMI is statistically controlled.

Table 6.2. Body composition of children with cerebral palsy (CP) and controls (con).

|                                      | All CP (n = 42)            | NACP (n = 18)              | ACP (n = 24)              | Con (n = 42) |
|--------------------------------------|----------------------------|----------------------------|---------------------------|--------------|
| %Fat                                 | 31.5 ± 10.7 <sup>1,2</sup> | 34.6 ± 11.6 <sup>1-3</sup> | 29.1 ± 9.5 <sup>2</sup>   | 24.4 ± 6.3   |
| Fat mass (kg)                        | 8.3 ± 5.9 <sup>2</sup>     | 9.3 ± 6.9 <sup>2,3</sup>   | 7.5 ± 5.0                 | 6.6 ± 2.7    |
| Fat-free mass (kg)                   | 16.0 ± 5.8 <sup>1,2</sup>  | 15.3 ± 5.4 <sup>1,2</sup>  | 16.5 ± 6.2 <sup>1,2</sup> | 20.3 ± 6.1   |
| Fat mass index (kg/ht <sup>2</sup> ) | 4.9 ± 3.0 <sup>1,2</sup>   | 5.5 ± 3.4 <sup>2,3</sup>   | 4.5 ± 2.2 <sup>2</sup>    | 3.6 ± 1.3    |
| Fat-free mass index                  | 9.8 ± 1.7 <sup>1,2</sup>   | 9.3 ± 1.5 <sup>1-3</sup>   | 10.2 ± 1.8 <sup>2</sup>   | 10.8 ± 1.4   |

NACP, nonambulatory CP; ACP, ambulatory CP. Values are means ± SD. <sup>1</sup>Different compared to controls,  $p < 0.05$ . <sup>2</sup>Different compared to controls when BMI was statistically controlled,  $p < 0.05$ . <sup>3</sup>Different compared to ACP when BMI was statistically controlled,  $p < 0.05$ .

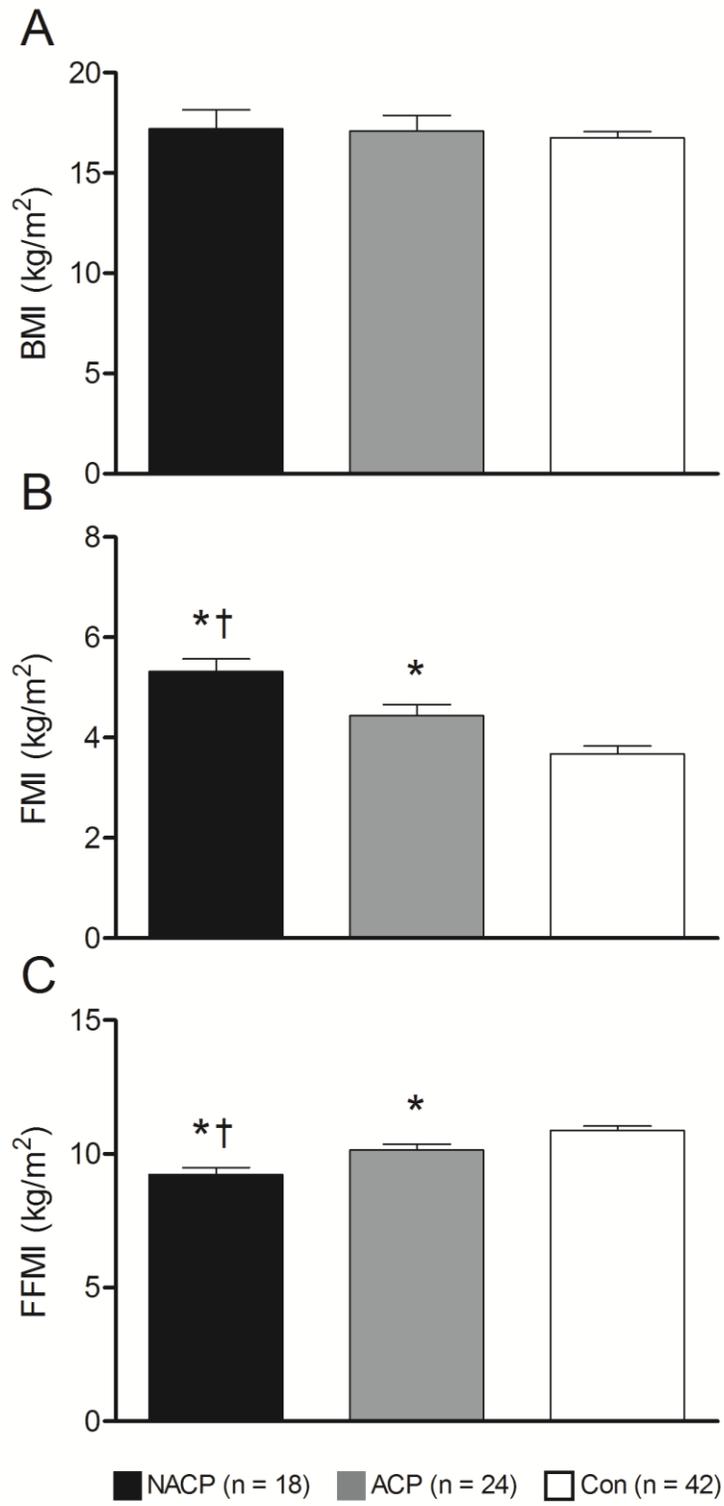


Figure 6.1. Bar graphs represent (A) body mass index (BMI), (B) fat mass index (FMI) statistically controlled for BMI and (C) fat-free mass index (FFMI) statistically controlled for BMI for nonambulatory children with cerebral palsy (NACP; black bar), ambulatory children with CP (ACP; gray bar) and typically developing children (Con; white bar). \*Different from controls,  $p < 0.05$ . †Different from ACP,  $p < 0.05$ .

#### 6.4.5 Estimating FMI and FFMI from BMI, Age, Sex and Ambulatory Status

Scatter plots in **Figure 2** demonstrate the relationship between BMI and FMI (A) and FFMI (B). BMI was a strong predictor of FMI and a moderately-strong predictor of FFMI in children with CP ( $r^2 = 0.83$  and  $0.49$ , respectively, both  $p < 0.001$ ) and a moderately-strong predictor of FMI and FFMI in controls ( $r^2 = 0.59$  and  $0.47$ , respectively, both  $p < 0.001$ ). The prediction of FMI ( $R^2 = 0.86$ ) and FFMI ( $R^2 = 0.66$ ) from BMI significantly increased (both  $R^2$  change,  $p < 0.05$ ) when age, sex and ambulatory status were included in the regression model, as shown in **Table 3** where height was estimated from knee height in nonambulatory children with CP for the calculation of BMI, FMI and FFMI (models 1 and 2, respectively). The prediction of FMI ( $R^2 = 0.84$ ) and FFMI ( $R^2 = 0.67$ ) from BMI increased ( $R^2$  change,  $p = 0.051$  and  $0.003$ , respectively) when age, sex and ambulatory status were included in the regression model, as shown in **Table 3** where height was estimated from forearm length in nonambulatory children with CP for the calculation of BMI, FMI and FFMI (models 3 and 4, respectively). All interaction terms between independent variables did not significantly contribute and were therefore not included in the model. Using the leave-one-out cross validation analysis procedure for children with CP, FMI from DXA and FMI predicted from BMI, age, sex and ambulatory status using model 1 (**Table 3**) were strongly correlated ( $r^2 = 0.81$ ,  $p < 0.001$ ; **Figure 3A**) and FFMI from DXA and FFMI

predicted from BMI, age, sex and ambulatory status using model 2 (**Table 3**) were moderately correlated ( $r^2 = 0.54$ ,  $p < 0.001$ ; **Figure 3B**).

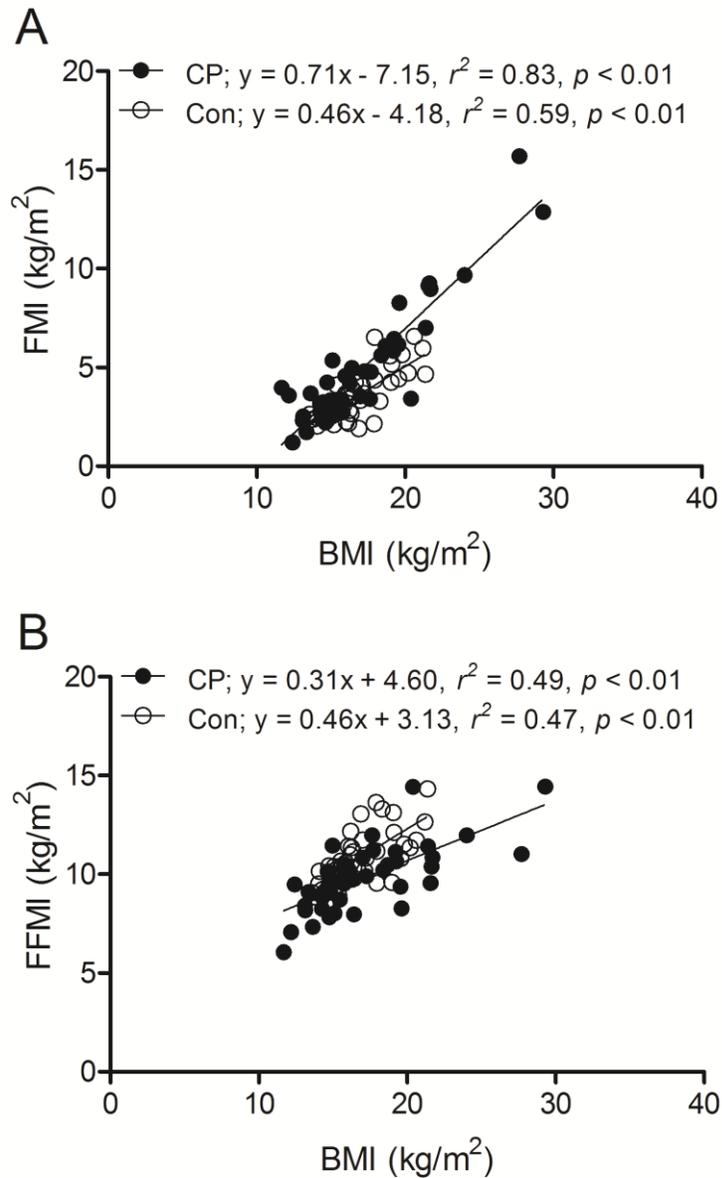


Figure 6.2. Scatter plot demonstrating (A) the relationships between body mass index (BMI) and fat mass index (FMI) and (B) the relationships between BMI and fat-free mass index (FFMI) in children with cerebral palsy (CP) and typically developing children (Con).

Table 6.3. Statistical models for predicting FMI and FFMI from DXA in children with cerebral palsy (CP) using BMI, age, sex and ambulatory status.

|   | Outcome Measure            | Coefficients | $\beta$ | t-value | SE    | $p$   | Model $R^2$ | Model adjusted $R^2$ |
|---|----------------------------|--------------|---------|---------|-------|-------|-------------|----------------------|
| 1 | *FMI (kg/m <sup>2</sup> )  | Intercept    | -7.223  | -7.274  | 0.993 | 0.000 | 0.862       | 0.847                |
|   |                            | BMI          | 0.736   | 14.572  | 0.051 | 0.000 |             |                      |
|   |                            | Age          | -0.111  | -1.352  | 0.082 | 0.185 |             |                      |
|   |                            | Female       | 0.546   | 1.327   | 0.412 | 0.193 |             |                      |
|   |                            | NACP         | 0.818   | 1.993   | 0.411 | 0.054 |             |                      |
| 2 | *FFMI (kg/m <sup>2</sup> ) | Intercept    | 3.949   | 4.490   | 0.880 | 0.000 | 0.655       | 0.618                |
|   |                            | BMI          | 0.261   | 5.839   | 0.045 | 0.000 |             |                      |
|   |                            | Age          | 0.221   | 3.026   | 0.073 | 0.004 |             |                      |
|   |                            | Female       | -0.383  | -1.050  | 0.365 | 0.301 |             |                      |
|   |                            | NACP         | -1.066  | -2.931  | 0.364 | 0.006 |             |                      |
| 3 | †FMI (kg/m <sup>2</sup> )  | Intercept    | -6.837  | -6.577  | 1.040 | 0.000 | 0.836       | 0.818                |
|   |                            | BMI          | 0.694   | 13.259  | 0.052 | 0.000 |             |                      |
|   |                            | Age          | -0.069  | -0.824  | 0.084 | 0.415 |             |                      |
|   |                            | Female       | 0.453   | 1.079   | 0.420 | 0.288 |             |                      |
|   |                            | NACP         | 0.931   | 2.201   | 0.423 | 0.034 |             |                      |
| 4 | †FFMI (kg/m <sup>2</sup> ) | Intercept    | 3.791   | 4.243   | 0.894 | 0.000 | 0.668       | 0.632                |
|   |                            | BMI          | 0.284   | 6.312   | 0.045 | 0.000 |             |                      |
|   |                            | Age          | 0.192   | 2.664   | 0.072 | 0.011 |             |                      |
|   |                            | Female       | -0.327  | -0.905  | 0.361 | 0.372 |             |                      |
|   |                            | NACP         | -1.158  | -3.186  | 0.364 | 0.003 |             |                      |

FMI, fat mass index (kg/m<sup>2</sup>); FFMI, fat-free mass index (kg/m<sup>2</sup>); NACP, nonambulatory children with CP. Height was used from standing height for ambulatory children with CP and controls and estimated from \*knee height or †forearm length for nonambulatory children with CP. Age is in years; Female = 1; NACP = 1. All models are significant,  $p < 0.001$ ,  $n = 42$ .

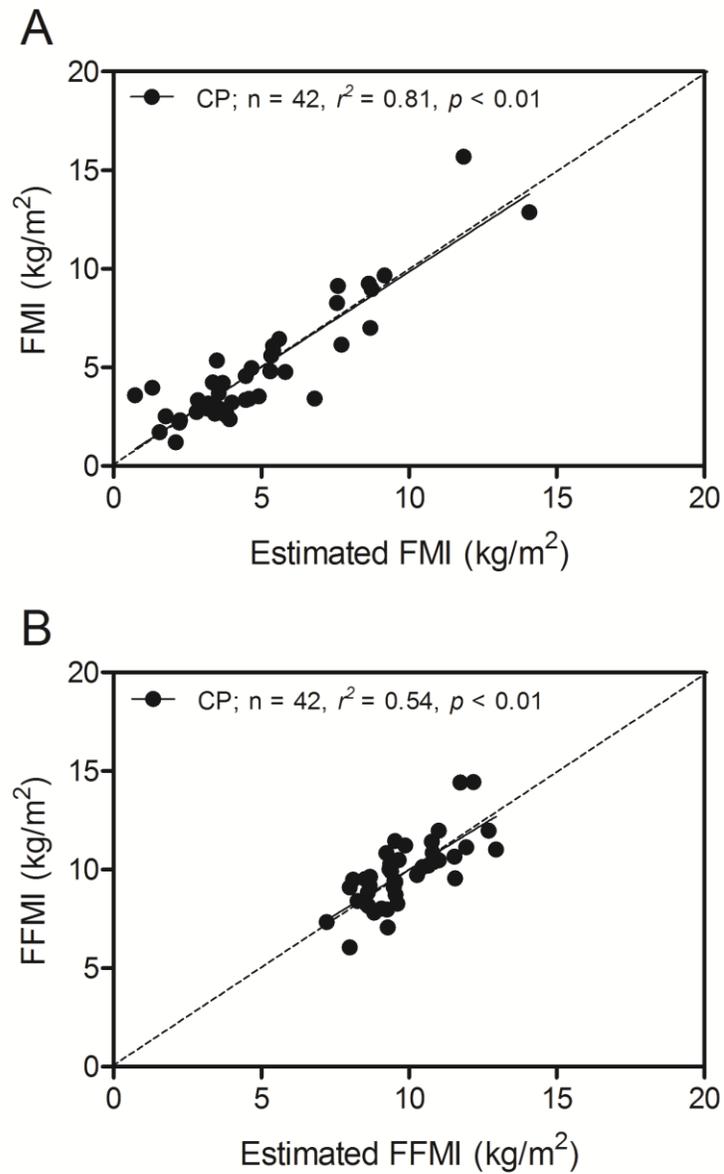


Figure 6.3. Scatter plot comparing the measured values to the estimated values of (A) fat mass index (FMI) and (B) fat-free mass index (FFMI) in children with cerebral palsy (CP) using multiple linear regression and body mass index, age, sex and ambulatory status and the models shown in Table 3. The dotted diagonal line represents the line of identity.

## 6.5 Discussion

To our knowledge, this is the first study to examine the relationship between BMI and its fat and fat-free equivalents (i.e., FMI and FFMI) in children with CP. The main finding is that FMI is higher and FFMI is lower for a given BMI in children with CP compared to typically developing children. Although the differences are more profound in nonambulatory than ambulatory children with CP, they are present in both groups. The result suggests that there is a misclassification of overweight/obesity status based on BMI in children with CP. The finding is problematic because chronic diseases often find their roots in childhood and obesity-related complications, such as metabolic syndrome [30] and cardiovascular-related mortality [31], are higher in adults with CP compared to the general population. Moreover, there is a high multi-morbidity prevalence in adults with CP that is related to obesity and higher levels of motor impairment [32]. Therefore, there is a need to more accurately assess overweight/obesity status in children with CP to better evaluate body composition types that may accelerate chronic disease progression. Fortunately, in the present study, we also developed and validated statistical models that more accurately estimate FMI and FFMI by BMI in children with CP.

Consistent with the present study, most studies have reported that BMI is not different in children with CP when compared to typically developing children [7, 13, 15]. In addition, the BMI percentiles of children with CP in the present study were not different from the 50<sup>th</sup> age- and sex-based percentiles. However, there was a greater proportion of children with CP than typically developing children that fell into the underweight category. This was observed despite the exclusion of obese children from the matched sample of typically developing children. The greater proportion of underweight children with CP compared to typically developing children is consistent

with a previous study [33]. The finding of an atypical distribution of weight classification and no difference in BMI compared to typically developing children, despite higher FMI and lower FFMI, suggests that BMI alone does not accurately reflect body composition or overweight/obesity status in children with CP because it is unable to distinguish between fat and fat-free tissues.

Although %Fat is viewed as a better marker of body composition and overweight/obesity status than BMI, it is flawed because it results from variation in fat tissue, fat-free tissue or both. This is demonstrated by the finding in the present study that children with CP had higher %Fat but no difference in fat mass compared to typically developing children. Instead, children with CP had lower fat-free mass, which is consistent with a previous study [7]. When body tissues were expressed relative to height using FMI and FFMI, children with CP had a higher proportion of fat and a lower proportion of fat-free tissue than typically developing children. Understanding this unusual body composition profile in children with CP is clinically important because, based on previous findings, %Fat has been recommended for routine use to assess their body composition [33].

BMI was strongly related to FMI and moderately-to-strongly related to FFMI in children with CP. These relationships were strengthened when age, sex and ambulatory status were included in the models with 86 % of the variance in FMI and 66 % of the variance in FFMI explained. The model predicting FMI cross-validated very well, as indicated by the strong relationship between the measured and predicted values ( $r^2 = 0.81$ ). Although the model predicting FFMI was not as accurate, it still cross-validated reasonably well, as indicated by the moderately-strong relationship between the measured and predicted values ( $r^2 = 0.54$ ).

The present study has several strengths. First, we compared body composition of children with CP and typically developing children using FMI and FFMI. Previous studies that reported on the absolute mass of fat and fat-free tissue in children with CP did not capture the proportion of these tissues because they did not account for their shorter stature compared to their typically developing peers. Moreover, fat-free mass may be accruing at a slower rate than fat mass in children with CP, which would go unnoticed when using %Fat to determine body composition. Therefore, FMI and FFMI may be better indicators of body composition than BMI and %Fat because they are expressed relative to the individual's height and are independent of the other tissue. Second, because the differences in body composition were more pronounced in nonambulatory than ambulatory children with CP compared to typically developing children, statistical models need to consider ambulatory status. Moreover, the independent predictors in the model are measures that are easily attainable during routine clinical visits or visits pertaining to research study participation. Determining height and weight for BMI, age, sex and ambulatory status do not require expensive equipment, accessibility to specialized facilities, rigorous training or exposure to radiation. Lastly, all children underwent DXA scans using the same scanner and software. It can be difficult to acquire an adequate sample of children with a clinical condition. This may lead to merging of data from different sites or comparing data to reference studies. Both scenarios pose the risk of differences in machinery, software or techniques used to acquire the data which can influence comparisons [34].

The limitations of this study must be discussed. First, markers of cardiometabolic health were not assessed. Although FMI and FFMI thresholds that discriminate metabolic syndrome have been introduced for older children and young

adults (12 to 20 years) [35], studies are needed to determine appropriate thresholds for younger children. Second, it is unknown if the statistical models developed in the current study can be used to monitor changes in body composition of children with CP due to growth, as well as surgery, treatment or nutritional changes. Children with severe cases of CP may be enterally-fed which can significantly effect growth [36], we were unable to determine. Unfortunately, the effect of enteral feeding on the BMI to FMI and BMI to FFMI relationships could not be assessed in the present study due to the small number of children who were enterally fed. Third, measurement of total height is difficult in children with CP, especially in nonambulatory children. In the present study, total height was estimated from knee height and forearm length in nonambulatory children with CP. Although there was very good agreement between height estimated from these two approaches ( $r = 0.91$  and no difference in values,  $p = 0.30$ ), models that estimate FMI and FFMI from BMI using the different height estimates for nonambulatory children with CP are presented in **Table 3**. Lastly, there is controversy surrounding the use of FMI to classify overweight/obesity status in children [37, 38] because it is unclear if fat mass scales to height squared, which is used in the BMI, FMI and FFMI calculations, or if it scales to height at another power (e.g., cubed). Ideally, the index chosen would not correlate with height. The differences in the strength of the correlation with height between fat mass expressed relative to height squared or height cubed vary depending on age [37]. Because the primary objective of the current study was to assess if BMI is capturing the proportion of the fat and fat-free components in children with CP, fat and fat-free mass were expressed relative to height squared for ease of comparison with BMI.

In conclusion, for a given BMI, children with CP had higher FMI and lower FFMI compared to typically developing children. Although the discrepancies were more profound in nonambulatory than ambulatory children with CP, they existed in both groups of children. Because many studies that document overweight/obesity status are done so using BMI [10-12, 39], the prevalence of overweight/obesity in children with CP may be even higher than reported previously. Importantly, this study provides validated statistical models to estimate FMI and FFMI from inexpensive, routine and easily attainable measures of BMI, age, sex and ambulatory status to provide a more accurate assessment of body composition in children with CP.

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### Author contributions

The author's responsibilities were as follows- CMM and DGW: designed the research; DGW and CMM: conducted the research; DGW, RTP and CMM: analyzed data; DGW and CMM: wrote paper; all authors: reviewed the manuscript, had primary responsibility for final content and approved the final manuscript. None of the authors reported a conflict of interest.

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## Chapter 7

### CONCLUDING SUMMARY

The overall objective of my dissertation project was to examine the local and global fat profile in children with spastic CP and to determine how fat distribution relates to muscle strength and physical activity. A brief summary of the results from the specific aims are below.

**Summary of specific aims 1 and 2:** Compared to typically developing children, ambulatory children with spastic CP had an underdeveloped musculoskeletal system, with a unique emphasis on the thinner medial portion of the tibial shaft, and lower estimates of bone strength. This underdeveloped musculoskeletal profile was accompanied by a high degree of fat infiltration of the skeletal depots (i.e., subfascial, intermuscular and intramuscular) and within the tibia bone marrow, but there were no group differences in total or subcutaneous fat of the leg. When the individual muscle profile of the leg was examined, ambulatory children with spastic CP had smaller muscles and higher intramuscular fat concentration of all muscles. Children with CP also had lower strength per unit volume of muscle which was partially explained by the higher intramuscular fat infiltration; although, to a small extent.

**Future directions:** Intramuscular fat is a composite of IMCL and EMCL with both depots having a unique role on the mechanical function of the muscle as well as local and systemic energy metabolism. If the higher intramuscular fat observed in ambulatory children with spastic CP is due to a higher partitioning of fatty acids towards IMCL, the muscle function may be impaired due to less contractile tissue for a given

muscle volume and the negative association between excess IMCL and myofibril transcription. Moreover, excess IMCL impedes insulin-mediated glucose uptake leading to peripheral insulin resistance. If the higher intramuscular fat observed in ambulatory children with spastic CP is due to a higher partitioning of fatty acids towards EMCL, the muscle function may be impaired due to a stiffer external environment for the muscle fiber to function in because of the association with EMCL and fibrotic tissue in the extramyocellular space. The role of EMCL on local or systemic energy metabolism has not been elucidated. Knowing whether the higher intramuscular fat observed in ambulatory children with spastic CP is due to a greater partitioning of fatty acids towards IMCL, EMCL or both, would allow for a better understanding of the health of the musculature.

**Summary of specific aim 3:** Compared to typically developing children, ambulatory children with spastic CP had higher trunk, abdominal and visceral FMI which was unrelated to the lower levels of physical activity. This may have been due to a basement effect where the levels of physical activity were too low to capture this relationship. While other factors may impact the higher accretion of fat within the visceral region for children with CP, the chronic levels of low physical activity certainly play a role.

**Future directions:** Determining the molecular profile of the visceral fat region in children with CP. Whether the higher visceral fat possesses a normal or higher inflammatory profile will give insight into the pathophysiology of excess fat accretion within this depot.

**Summary of specific aim 4:** There were no differences between BMI or age- and sex-based BMI percentile between children with spastic CP and typically

developing children. Moreover, BMI percentile was around 50 % for children with CP and typically developing children. However, for a given BMI, children with CP had higher FMI and lower FFMI for the whole body. While this existed for both nonambulatory and ambulatory children with CP compared to typically developing children, these differences were more pronounced in nonambulatory than ambulatory children with CP. This suggests a misclassification of weight status by BMI and BMI percentile. While many studies have reported that the overweight/obesity prevalence in children with CP is similar to that of the general population of children, these studies assessed weight status by using age- and sex-based BMI percentile. Therefore, the overweight/obesity prevalence is higher than reported. Importantly, we developed and validated statistical models that predict FMI and FFMI for children with CP using simple and easily obtainable measures, such as BMI, age, sex and ambulatory status.

**Future directions:** To determine the relationship of this unique body composition of high body fat and low body fat-free with markers of cardiometabolic disease risk. Moreover, to determine if our validated models to predict FMI and FFMI in children with CP can capture cardiometabolic health.

In conclusion, children with spastic CP are at a very high risk for early and accelerated development of obesity and obesity-related chronic diseases.

## Appendix A

### APPROVED INSTITUTIONAL REVIEW BOARD DOCUMENT



Nemours Office of Human Subjects Protection  
10140 Centurion Parkway North  
Jacksonville, FL 32256  
Phone: 904-697-4023 Fax: 904-697-4024

#### MEMORANDUM

DATE: February 27, 2012

TO: Freeman Miller, MD

FROM: Nemours Delaware IRB

STUDY TITLE: [115648-2] Effect of Botox and vibration on bone in children with cerebral palsy

IRB #: 115648

SUBMISSION TYPE: Response/Follow-Up

ACTION: APPROVED

APPROVAL DATE: January 26, 2012

EXPIRATION DATE: January 25, 2013

Thank you for your submission of Response/Follow-Up materials for this research study. Your initial submission received full review at the January 26, 2012 meeting and met all DHHS [and FDA] criteria for approval. The approval was contingent on the response to minor stipulations. Your response has received Expedited Review and is accepted. The above-referenced research study is approved.

The IRB has determined that:

- This is "Research involving greater than minimal risk but presenting the prospect of direct benefit to the individual subjects per 45CFR46.405 and 21CFR50.52".
- Parental Permission is required prior to initiation of any research procedures using only the most current IRB approved form(s) posted as a Board Document in IRBNet. All protocol documents, including Board approved documents are found in the "Study Designer" for each study in IRBNet.
- The IRB approved telephone script is required prior to initiation of any research procedure.
- The permission of one parent is sufficient. A person who is not a parent may not give permission without prior IRB review and approval.
- Assent of minors is required prior to initiation of any research procedures, using only the most current assent form(s) posted as a Board Document in IRBNet. If the investigator chooses to obtain assent, the form(s) listed below must be used and the minor's dissent must be honored.
- A signed copy of the Parental Permission/Informed Consent form must be included in the Nemours' medical record. Research data may also be included into the Nemours medical record.
- To continue, the research requires IRB review and approval on an annual basis. Otherwise, January 25, 2013 is the last day that research may be conducted. The Principal Investigator is responsible for the timely submission of the continuing review application. Please post this date on your research calendar.

Reviewed/approved documents in this submission:

- Application Form - Revised Application Form (UPDATED: 02/14/2012)
- Child Assent - Revised child assent (UPDATED: 02/14/2012)

- Cover Sheet - Cover letter - response to initial IRB review (UPDATED: 02/14/2012)
- Other - Data Safety Monitoring Board Charter (UPDATED: 02/13/2012)
- Other - Data Safety Monitoring Plan (UPDATED: 02/13/2012)
- Other - Edited verbal recruitment (UPDATED: 02/13/2012)
- Parental Permission Form - Parental permission form revised (UPDATED: 02/14/2012)
- Protocol - Revised protocol (UPDATED: 02/13/2012)

The IRB requires that a copy of the participant brochure: "Becoming A Research Volunteer" will be given to every individual enrolled in a research study. The PDF file for this document has been attached to this study as a Board Document.

**Investigator Agreement:** As the PI, you have agreed to assure that this research is conducted in compliance with Nemours policy and all applicable federal regulations and ICH standards, including, but not inclusive of:

- All research must be conducted in accordance with this approved submission. Any revision to approved materials must be approved by the IRB prior to initiation.
- Remember that informed consent/parental permission is a process beginning with a description of the study and insurance of participant understanding followed by a signed consent form. Informed consent must continue throughout the study via a dialogue between the researcher and research participant. Federal regulations require each participant receive a copy of the signed consent document.
- All serious and unexpected adverse events and unanticipated problems affecting participants must be reported promptly to the IRB according to NOHSP policy.
- All non-compliance issues or complaints regarding this study must be reported to the Director, NOHSP.
- All research records must be retained for a minimum of three years.
- A Closure Report must be submitted to the IRB when this protocol is completed.

If you have any questions, please contact Camille Varacchi at Nemours Al duPont Hospital for Children 1600 Rockland Road, ARB-Room 291, Wilmington, Delaware 19803, 302-651-6807 or [cvaracch@nemours.org](mailto:cvaracch@nemours.org).

Please include your study title and reference number in all correspondence with this office.

## Appendix B

### PERMISSIONS

06/08/17

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Jun 08, 2017

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